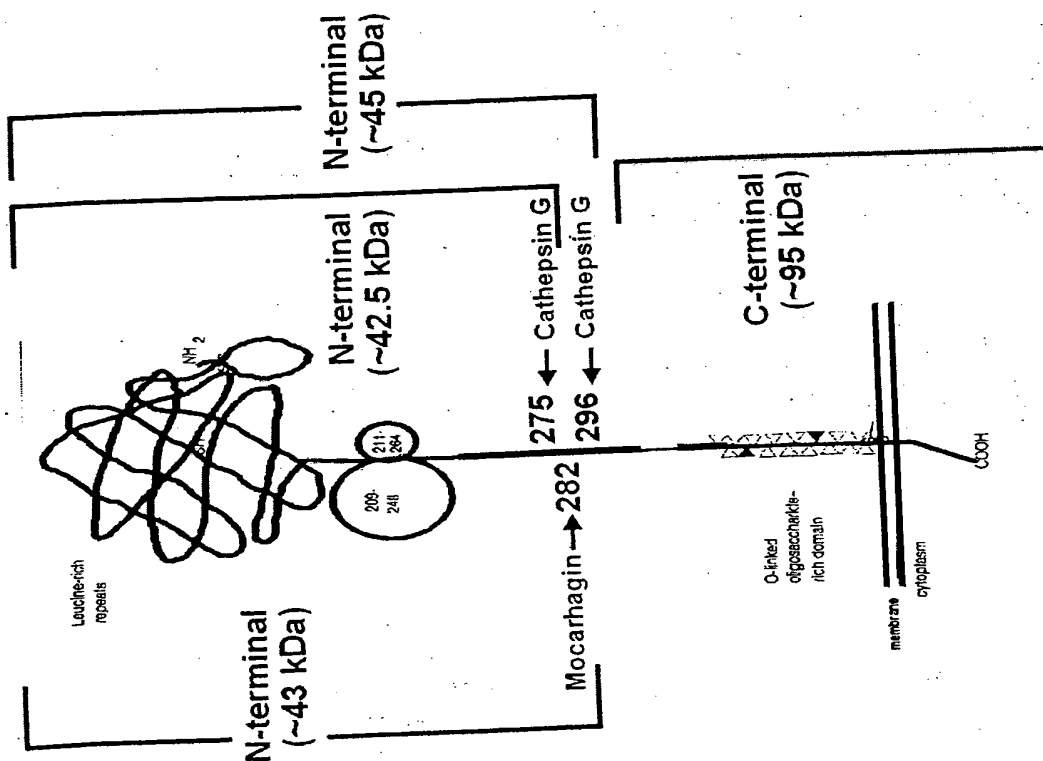




FIG. 1

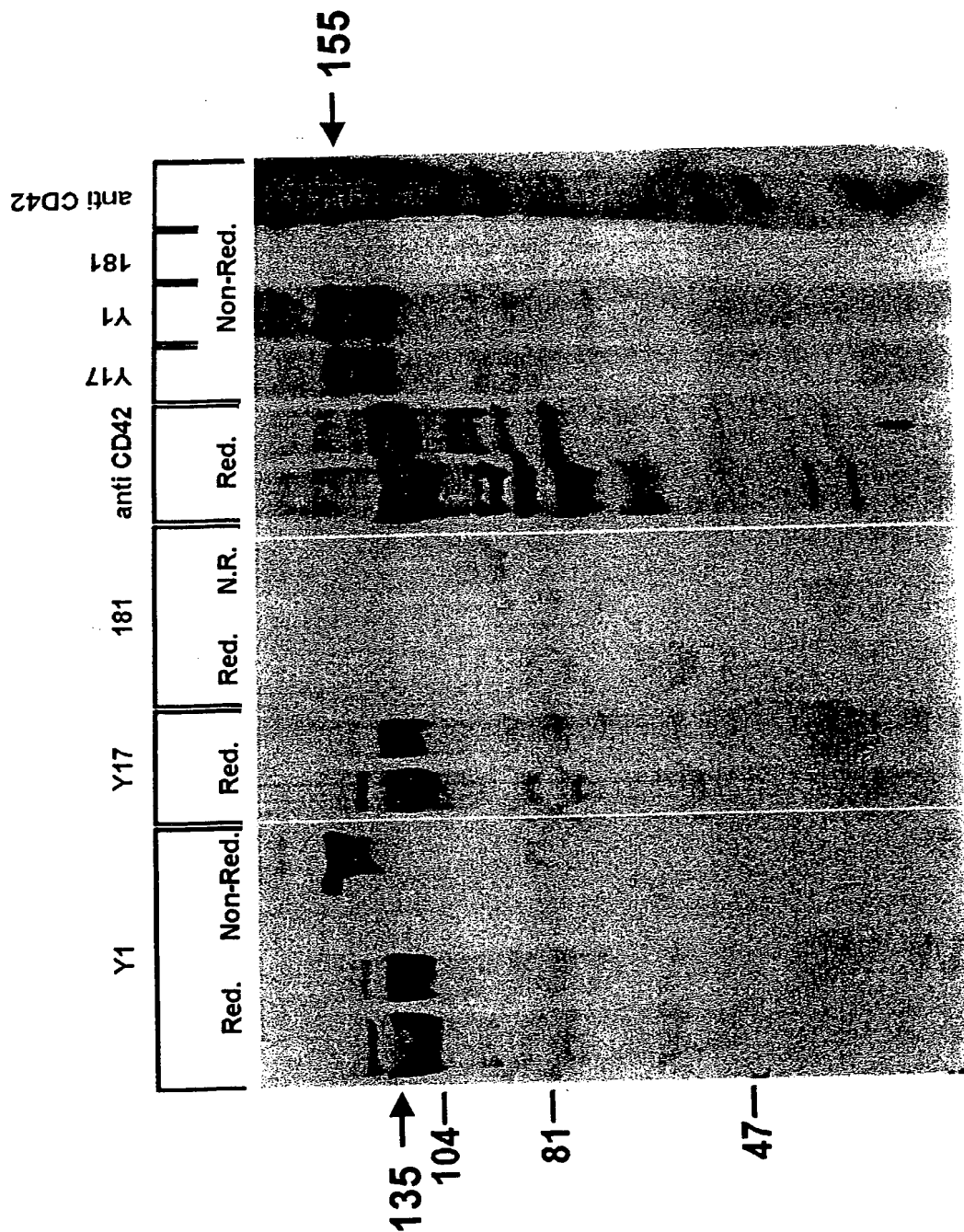
Cleavage sites of endoproteases on the a-chain of GPIb



#14

FIG. 2

Binding of Y1 and Y17 to platelets in reduced and non-reduced conditions





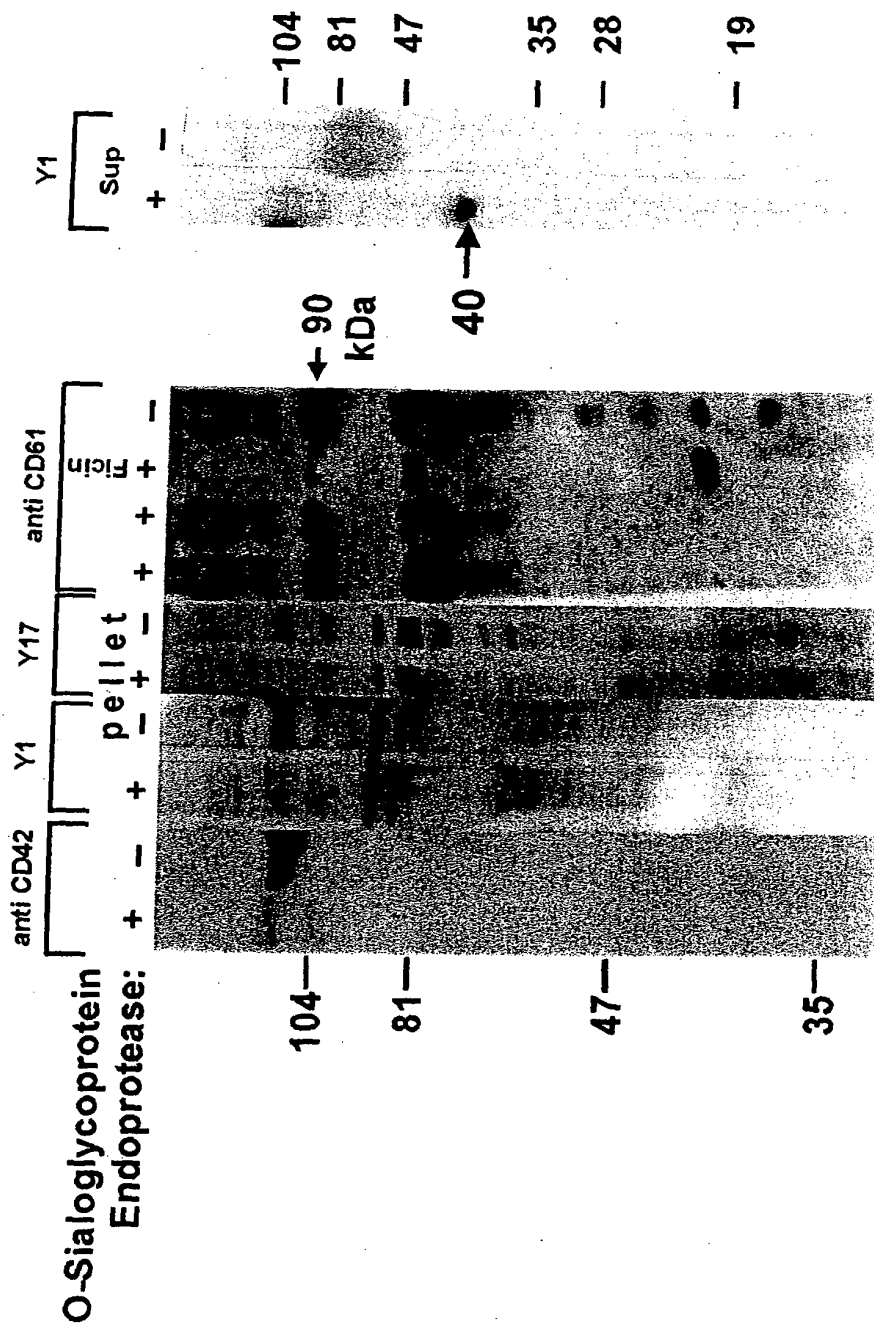
Characterization of Optimal Determinants for Binding of Y1 to It's Ligands

FIG. 3

	Platelets/GC	KG1/RP-HPLC #4
Rec: GP1b 1-340 GP1b 1-480	- -	
Glycanase: N N+O	+ +	+++ +++
Proteases: Mocarhagin O-Sialo Peptidase Ficin Trypsine Elastase	++ (~40kD) ++ (~40kD) - ++ (~40kD) ++ (~40kD)	- - - - ++
Sulfatase (Aryl)		-/+



Cleavage of platelets GPIb by O-Sialoglycoprotein abolishes binding of both Y1 and Y17



Y1 and Y17 binds similar glycosylated fragments after cleavage by O-Sialoglycoprotein Endoprotease

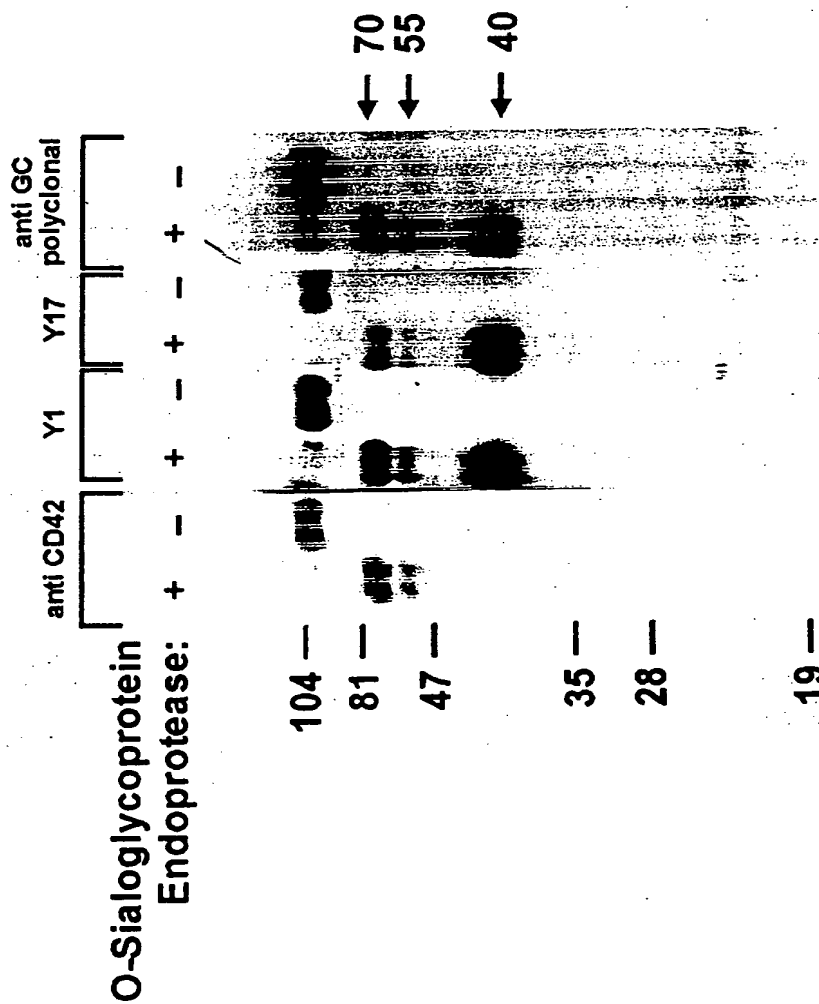
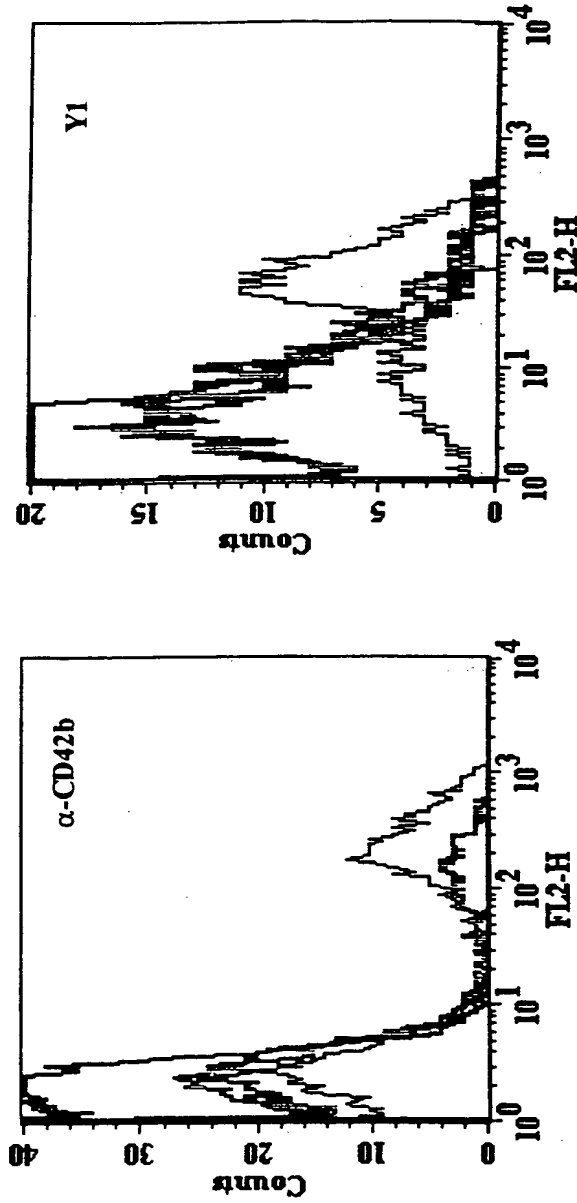


FIG. 5

Specific GPIIb Proteolysis Abolishes Y1 Binding to Platelets

FIG. 6



Key	Name	Parameter	G
	NON-TREATED PLATELETS		
---	O-SIALOGLYCOPROTEIN ENDO. (10 μ g/ml)		
---	O-SIALOGLYCOPROTEIN ENDO. (50 μ g/ml)		
---	FICIN (18 μ g/ml)		

Y1 binds N-terminal (His-1 - Glu 282) fragment of platelet GPIb after cleavage by mocarhagin

FIG. 7

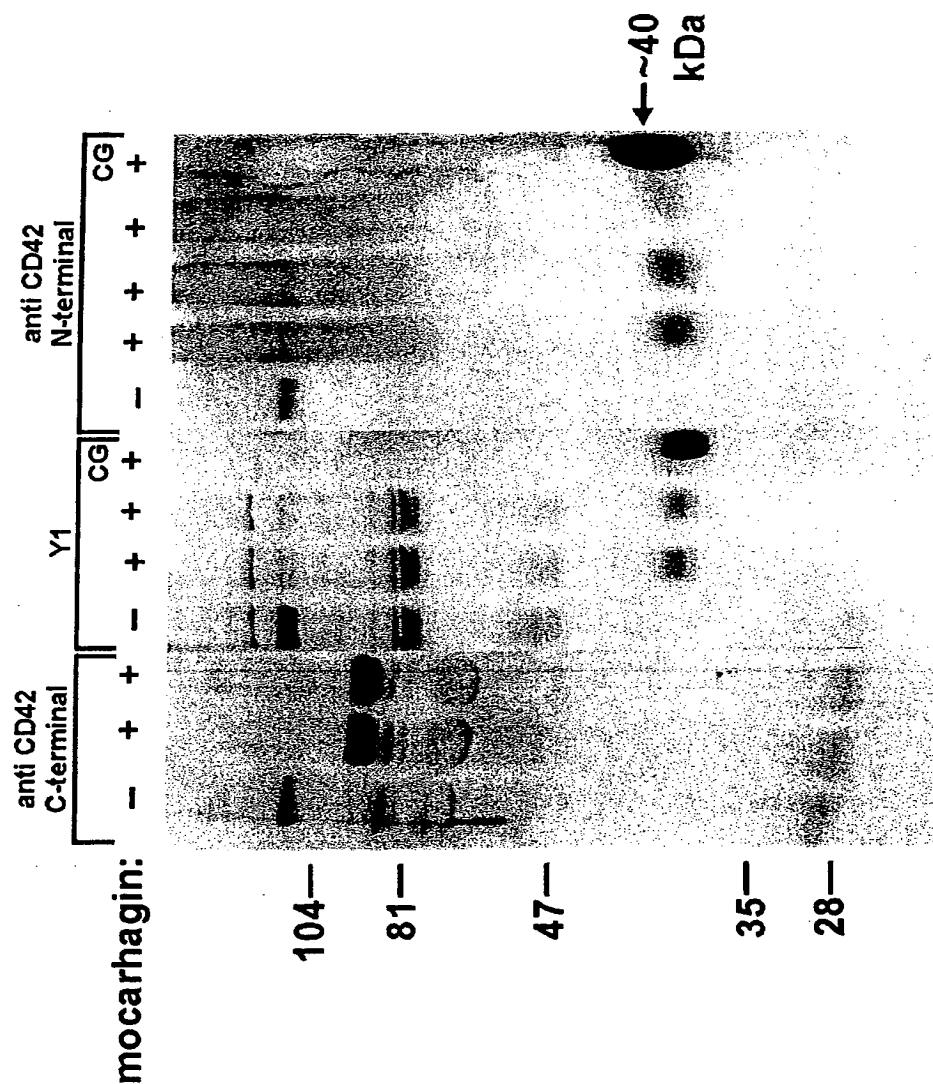


FIG. 8

Binding of Y1 and Y17 to glycolalycin after
 cleavage by mocarhagin

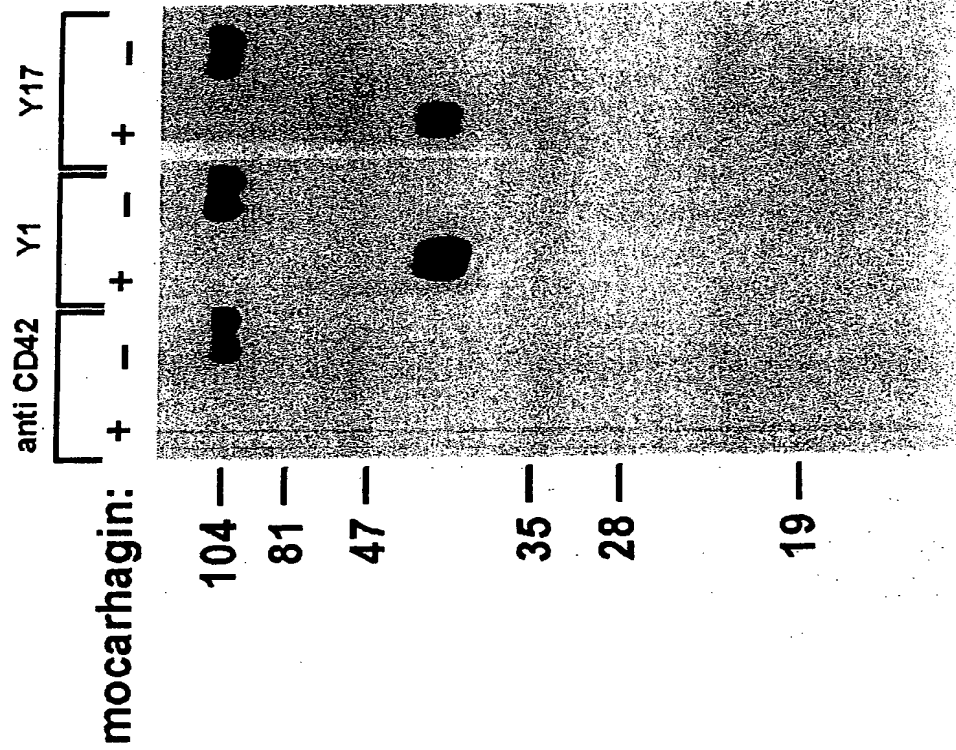
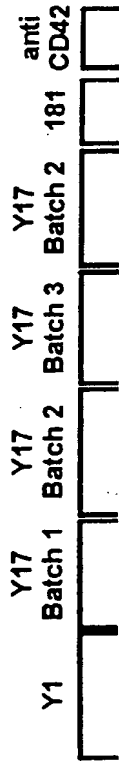


FIG. 9

Binding of Y1 and Y17 to platelets



104—

81—

47—

35—

28—

Y1 and Y17 bind glycoprotein similar after cleavage by Ficin

FIG. 10

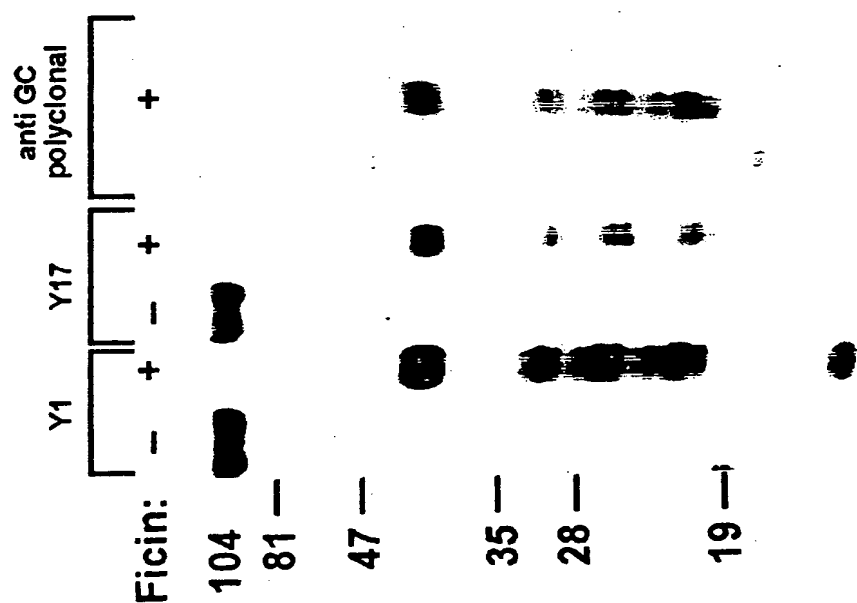
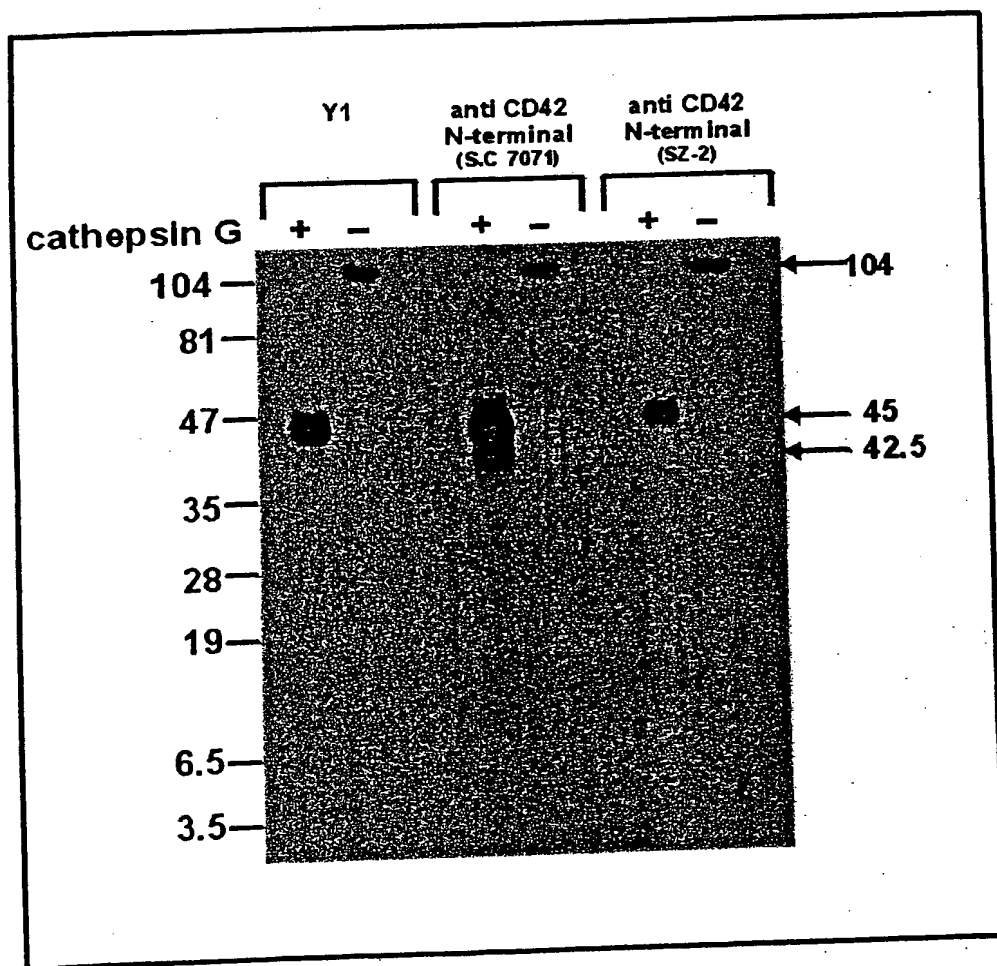


FIG. 11



Y1 and Y17 reacts with larger cathepsin G cleaved platelets GPIIb fragment

FIG. 12

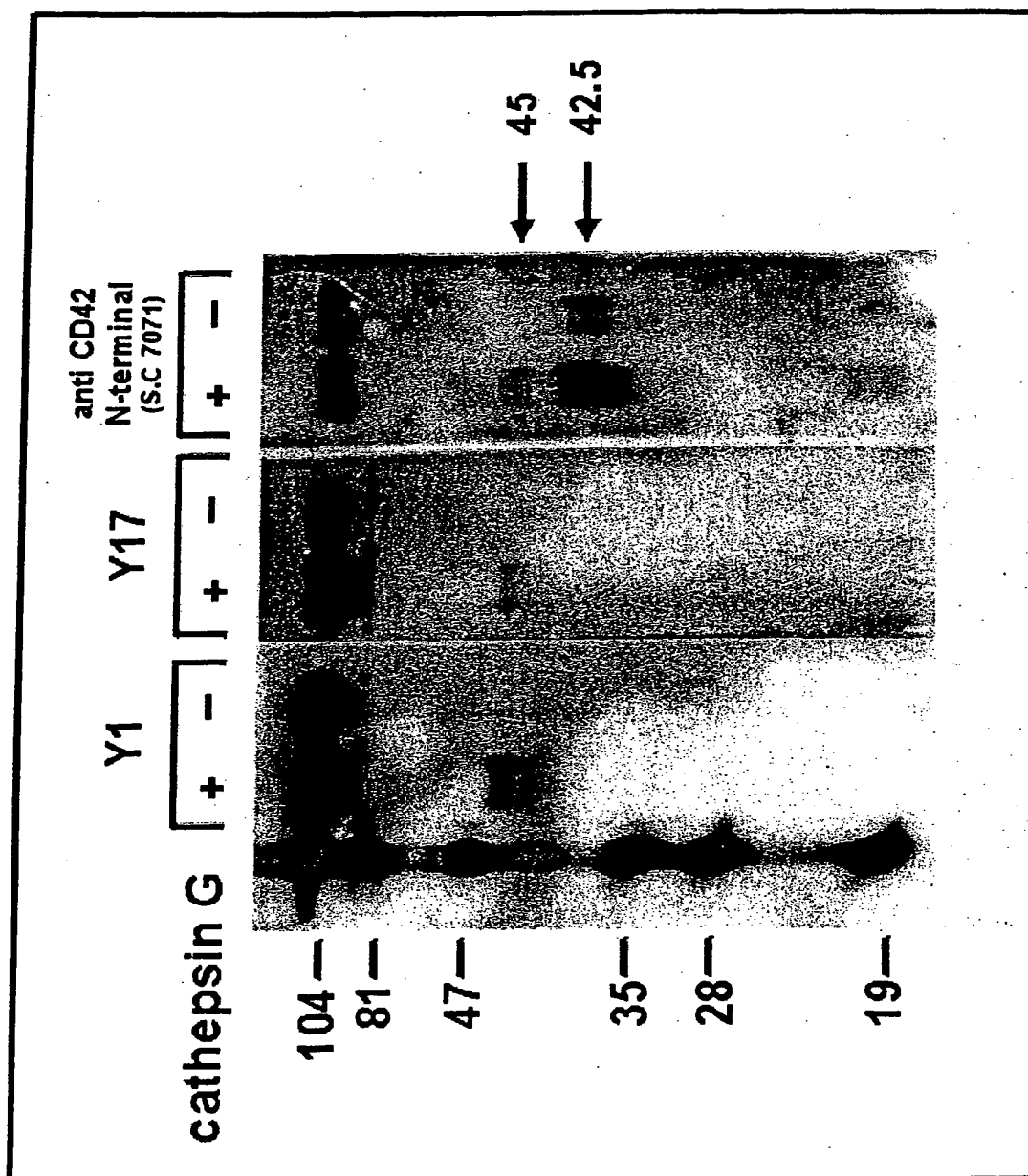
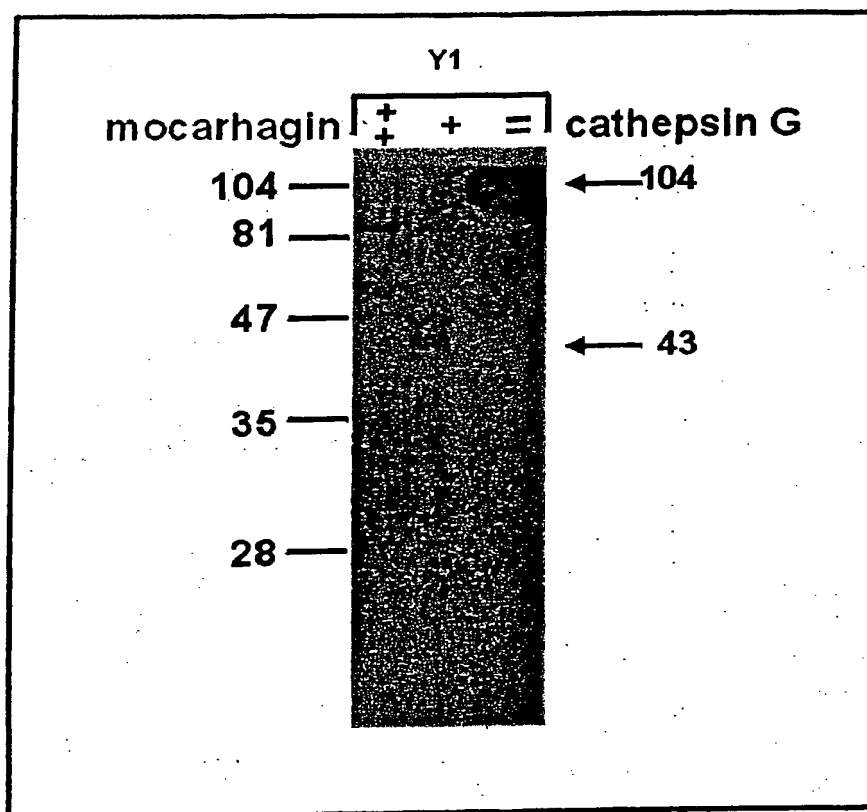




FIG. 13



Cleavage of washed platelets by mocarhagin and cathepsin G

FIG. 14

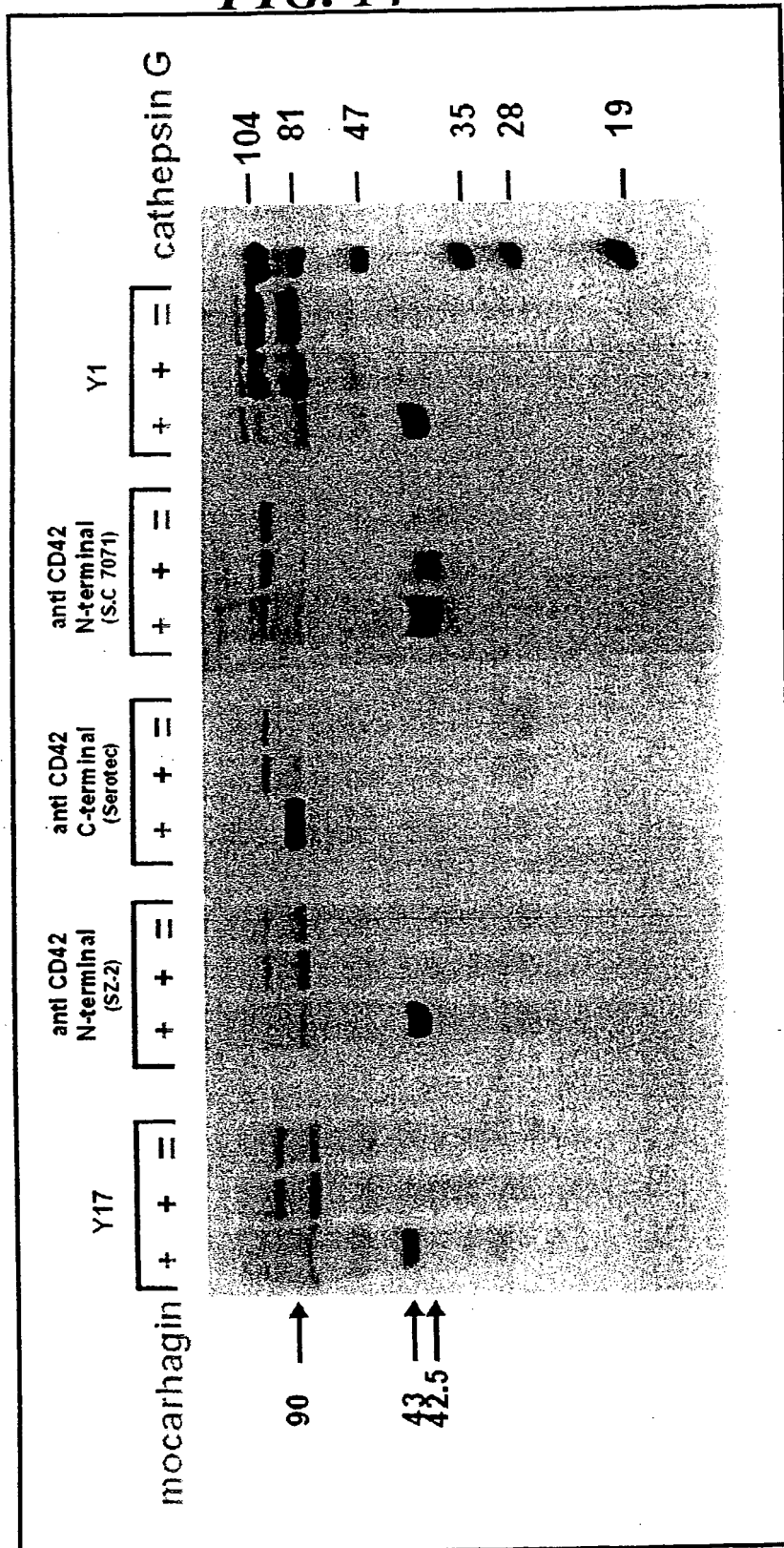


FIG. 15

**Influence of Y1-scFv on platelets agglutination in
washed platelets**

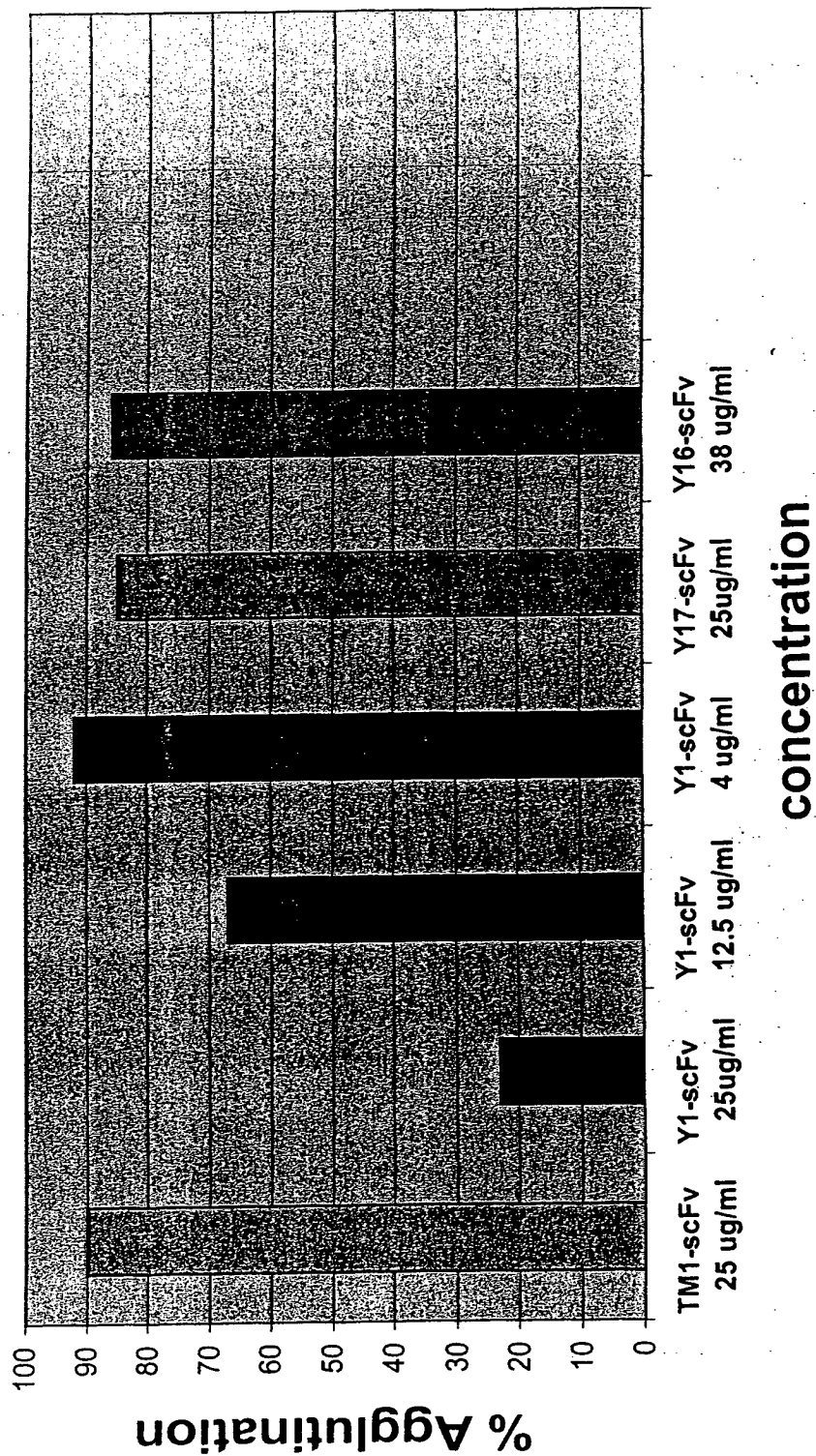




FIG. 16

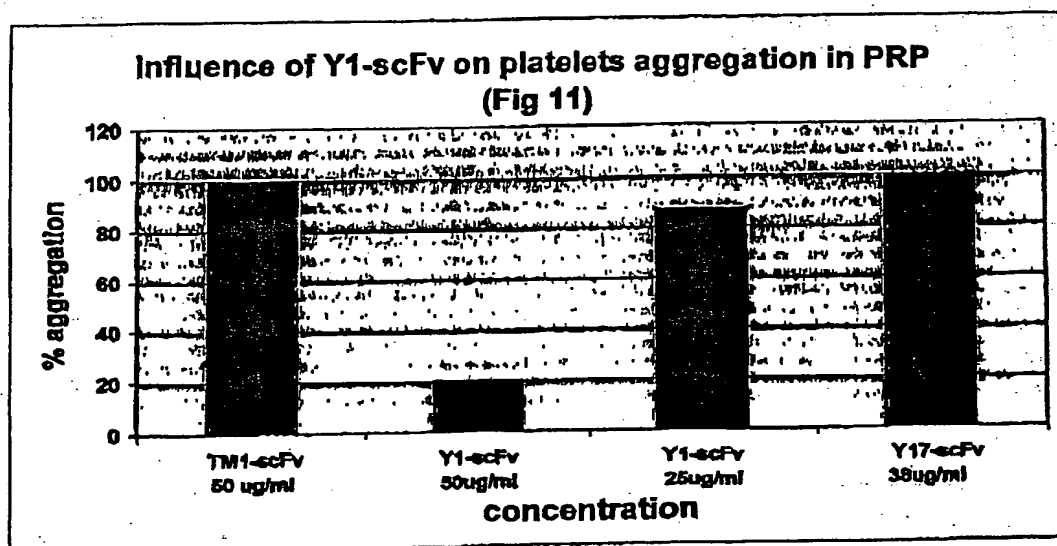


FIG. 18

Induction of platelet aggregation by Y1-IgG in PRP

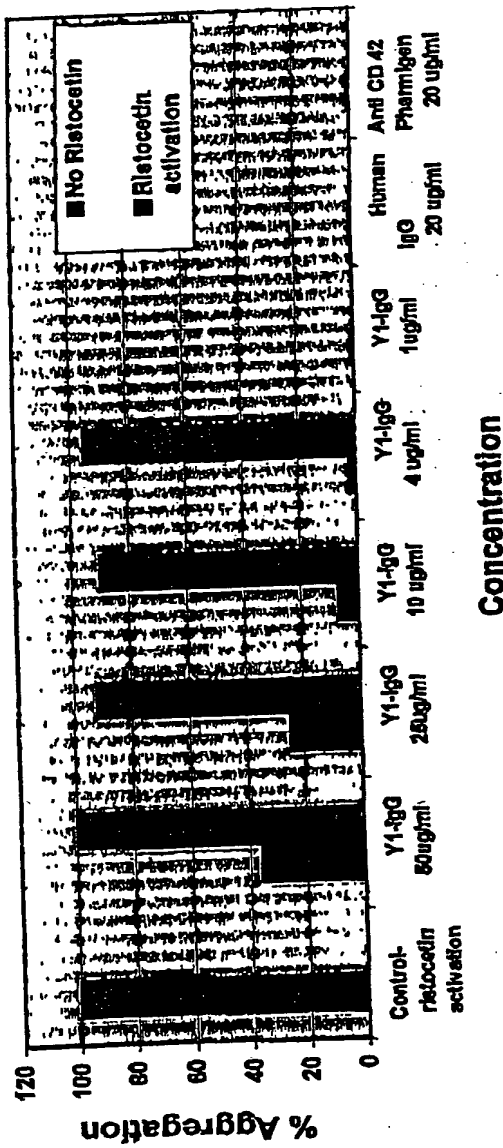
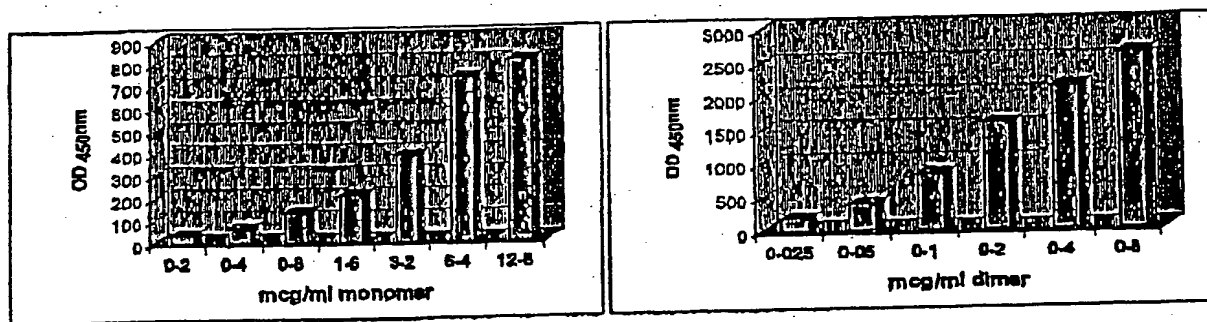




FIG. 19



Specificity of Binding of Y1 and α -CD42 (N1-19) to their Ligands

FIG. 20

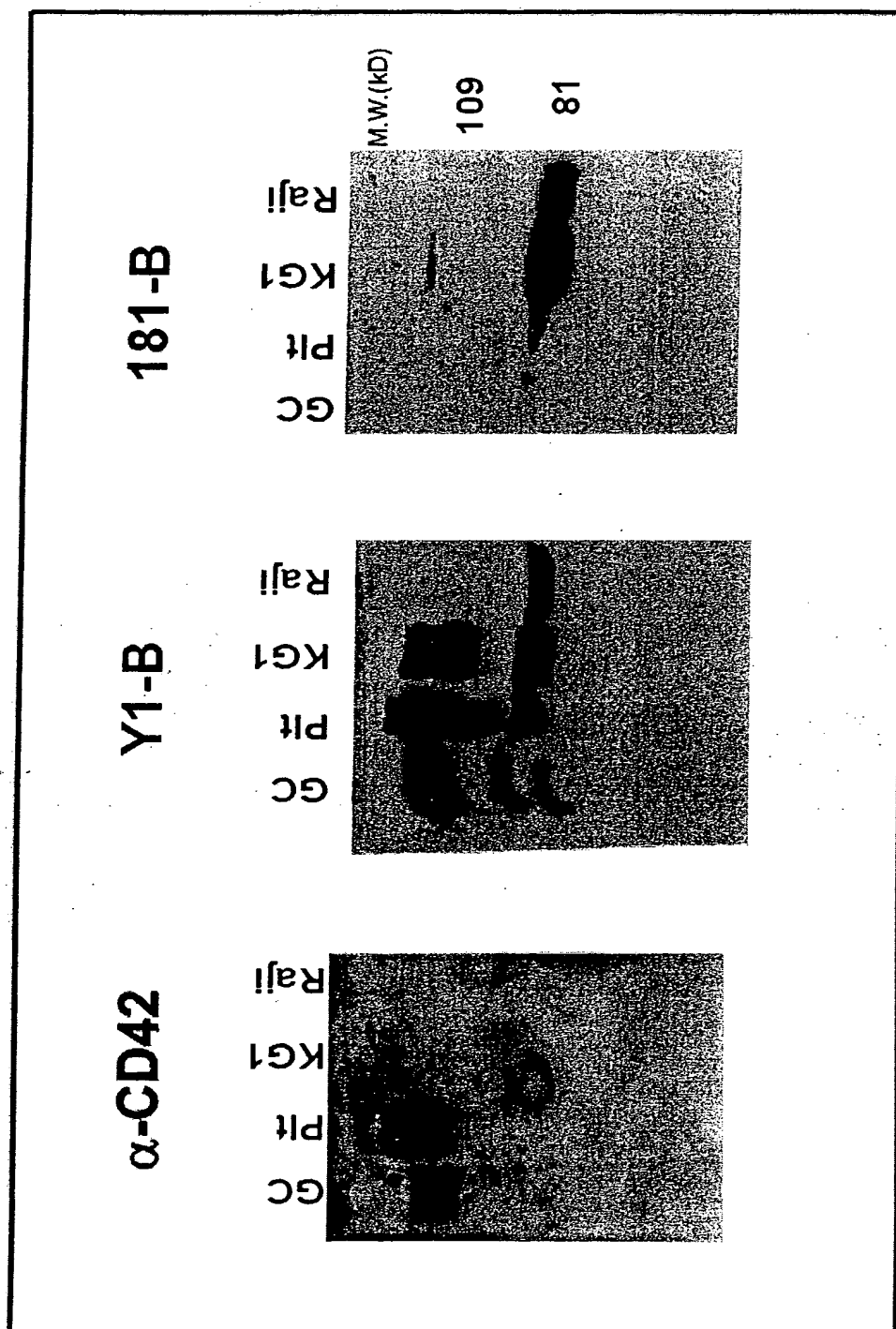
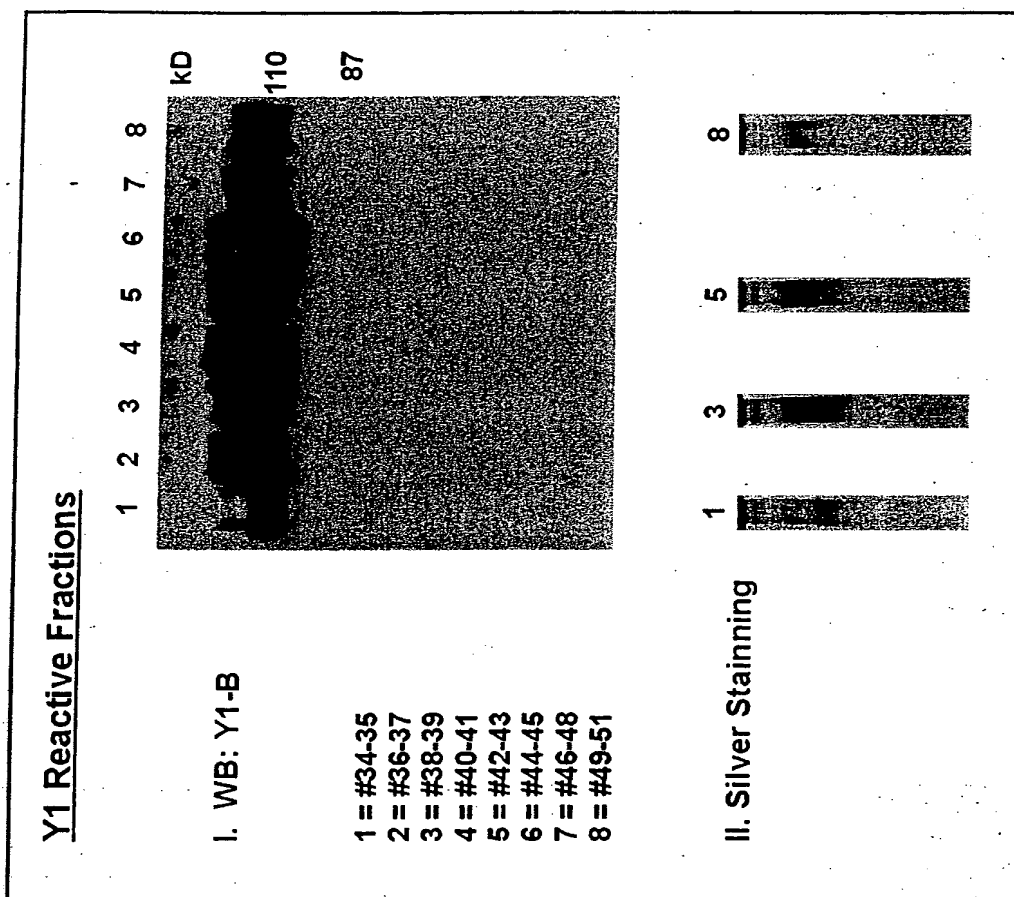


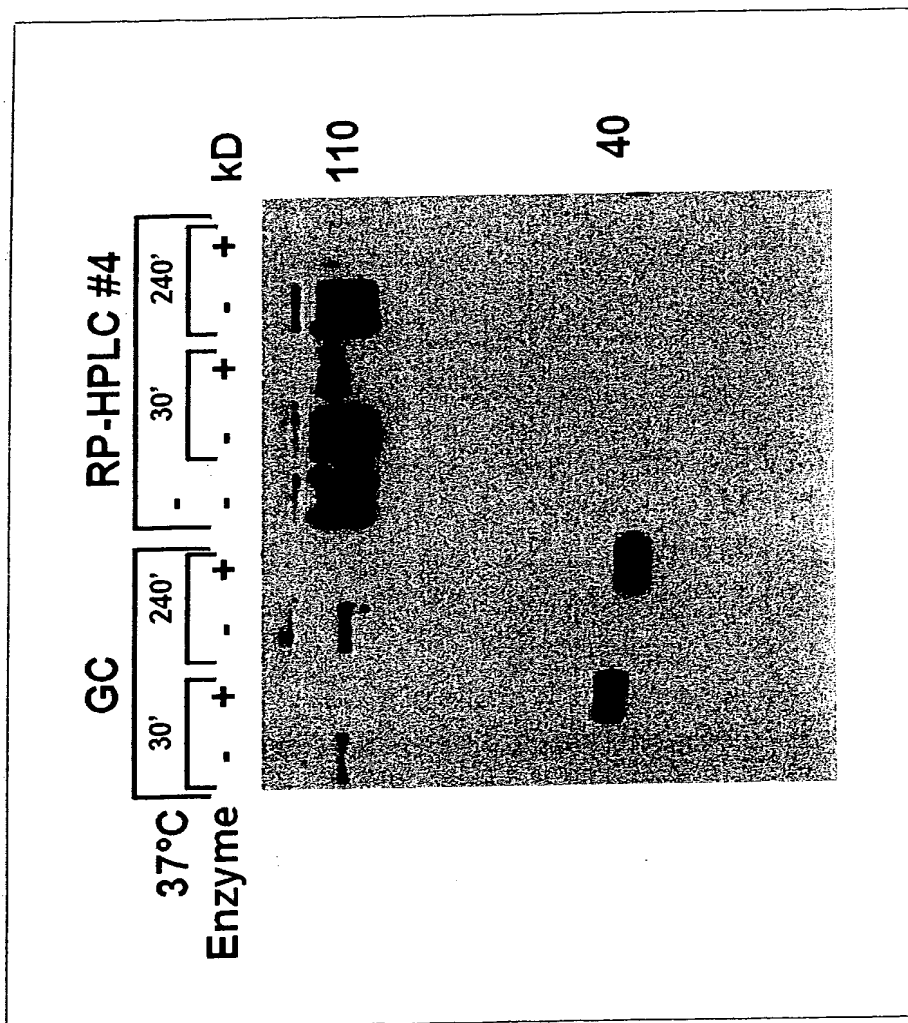
FIG. 21

Y1-Ligand from KG1 membranes following Immuno-Precipitation with Y1: Purification on RP-HPLC



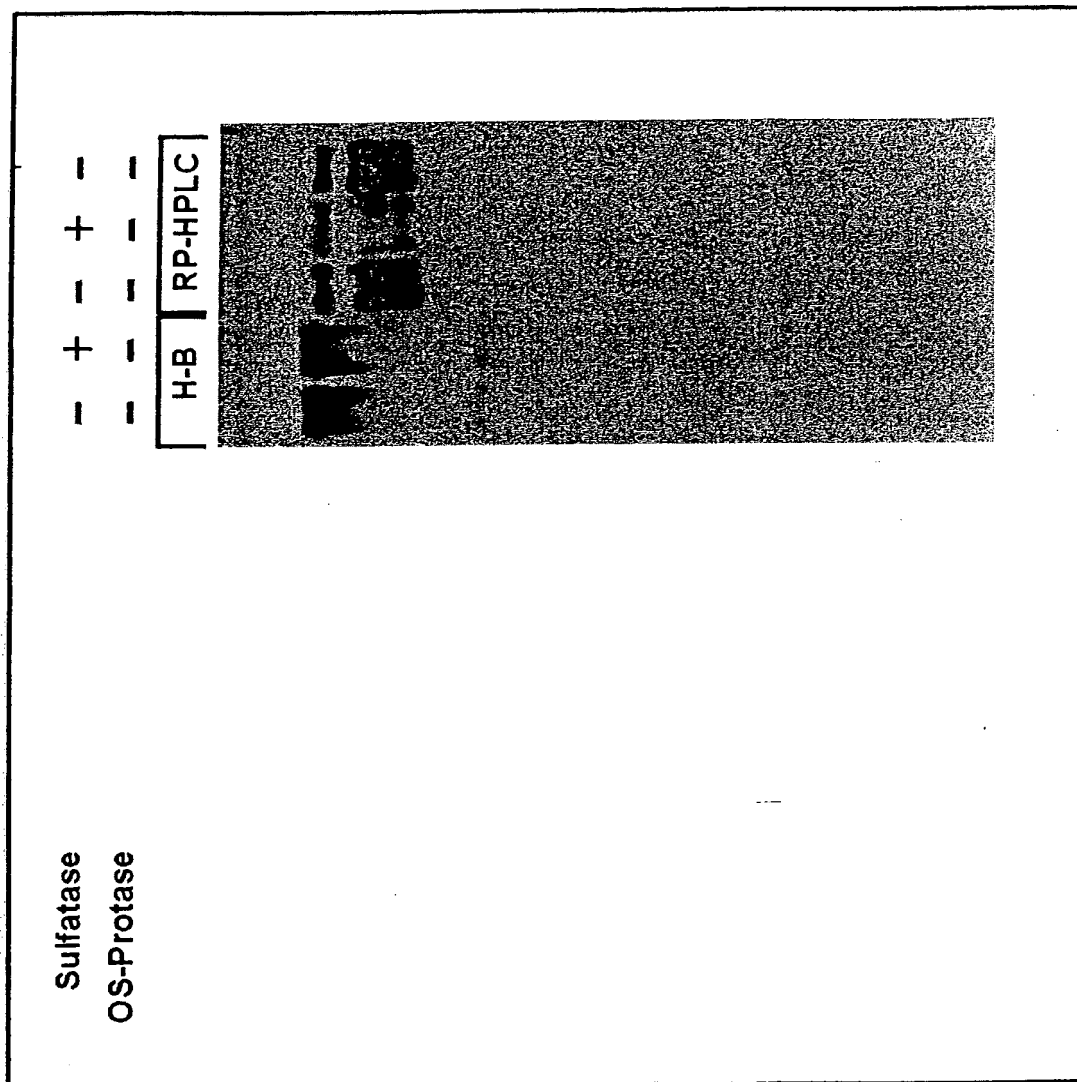
Effect of O-Sialo-Glycoprotein Endopeptidase on Y1 Binding

FIG. 22



Effect of Aryl-Sulfatase on Binding of Y1: RP-HPLC(KG1) & H-B(Heparin-BSA)

FIG. 23



Specificity of Y1 Binding: Analysis by Immune Precipitation with Y1 and anti-PSGL-1

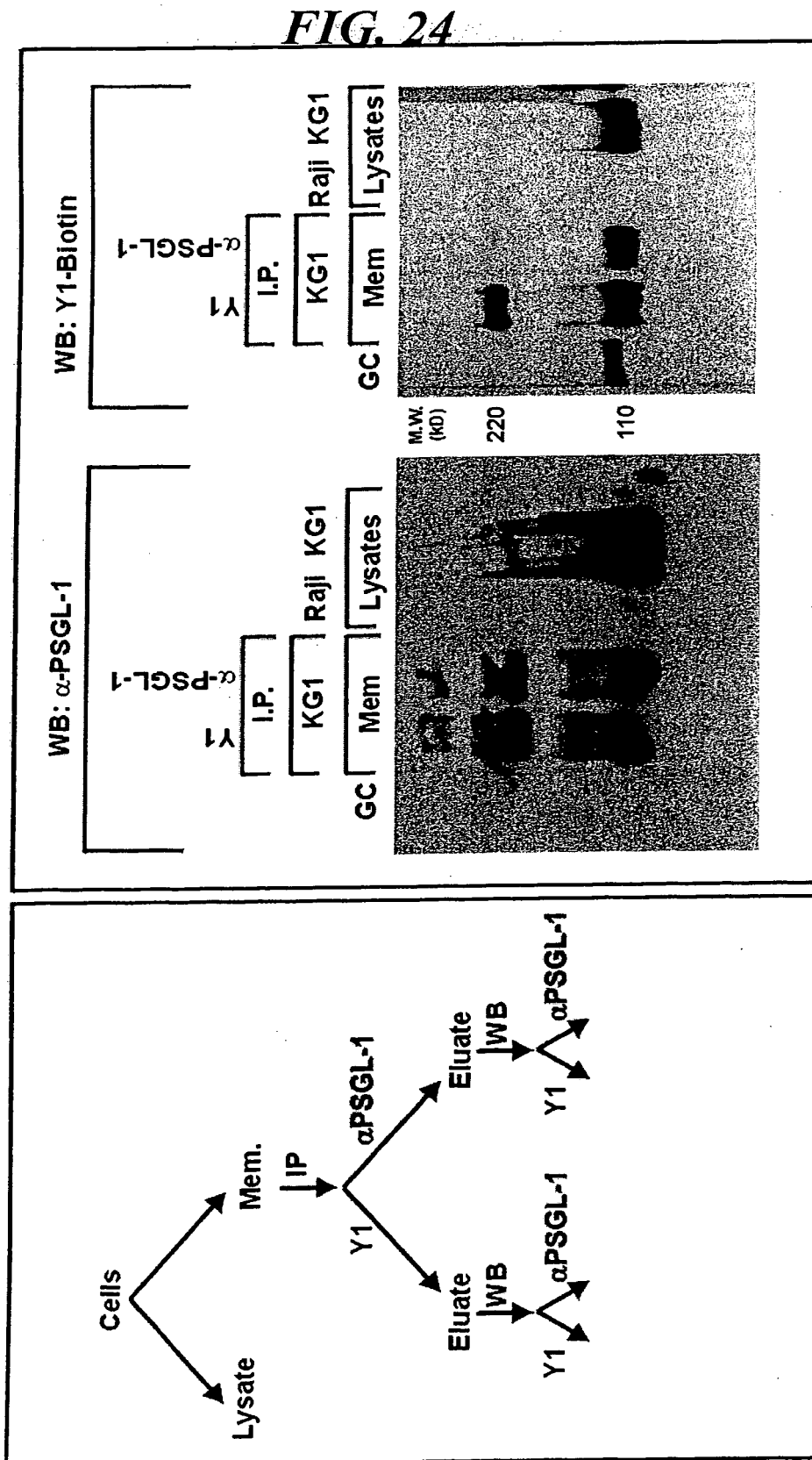


FIG. 25

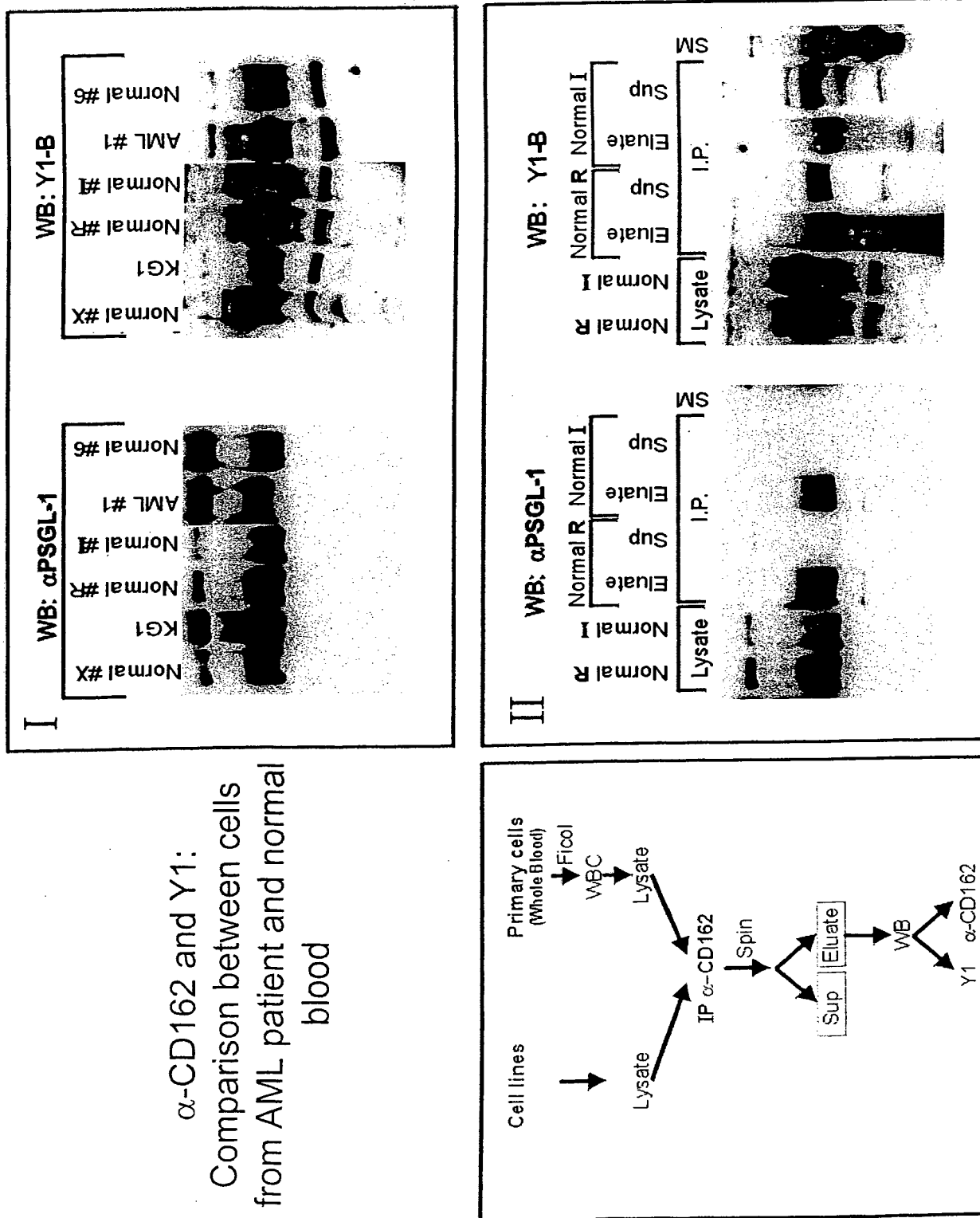
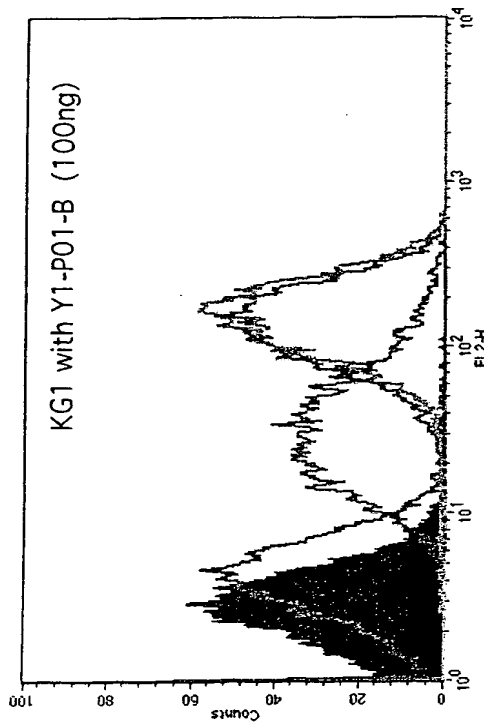
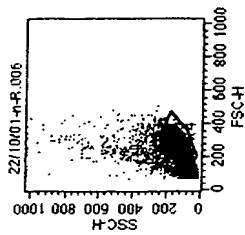
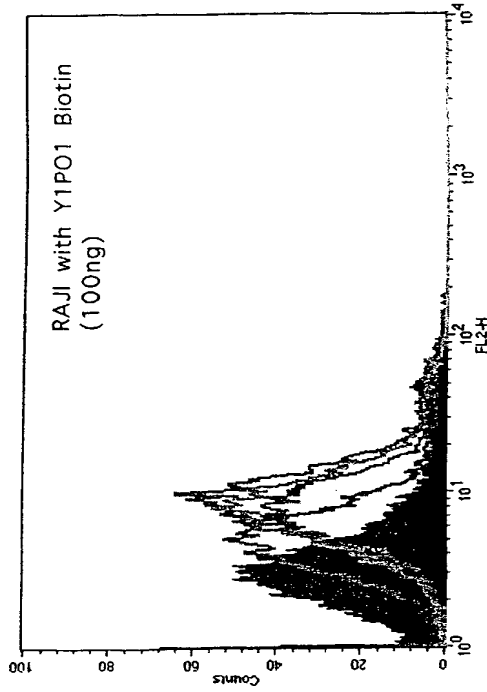
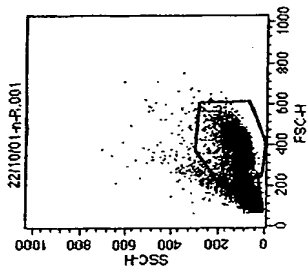


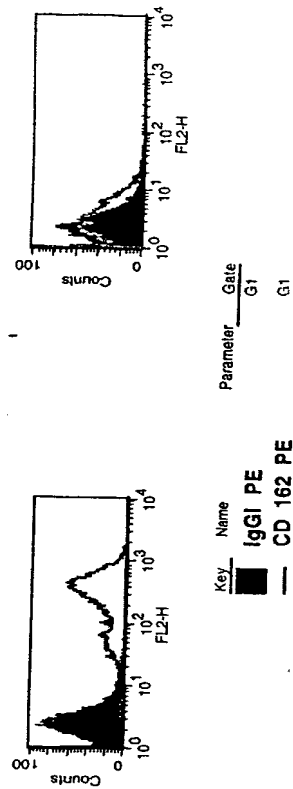


FIG. 26



Key	Name	Parameter	Gate
■	22101001-n-R.006	N01-B	
■	22101001-n-R.007	P01-B	
■	22101001-n-R.008	+KPL1	
■	22101001-n-R.009	+PL1	
■	22101001-n-R.010	+PL2	

Specificity of Y1 Binding: Analysis by FACS



- Binding of
 α PSGL1
 $(\alpha$ CD162/KPL1);
 competition
 with Y1-IgG

FIG. 27

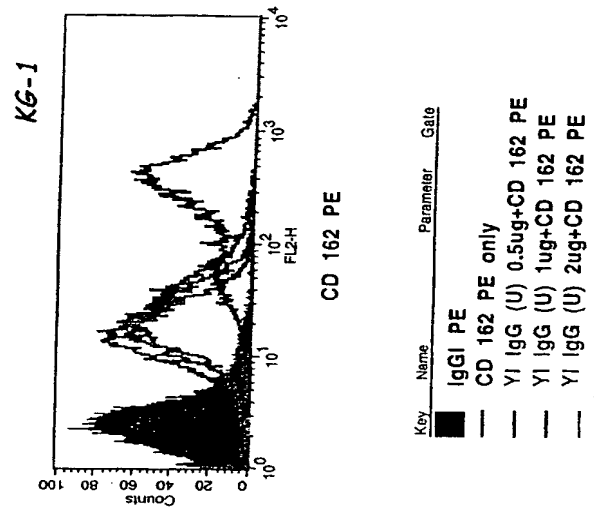




FIG. 28

Specificity of Y1 Binding: Analysis by FACS

- Binding of Y1-IgG; competition with α PSGL-1 (CD162 /KPL1)

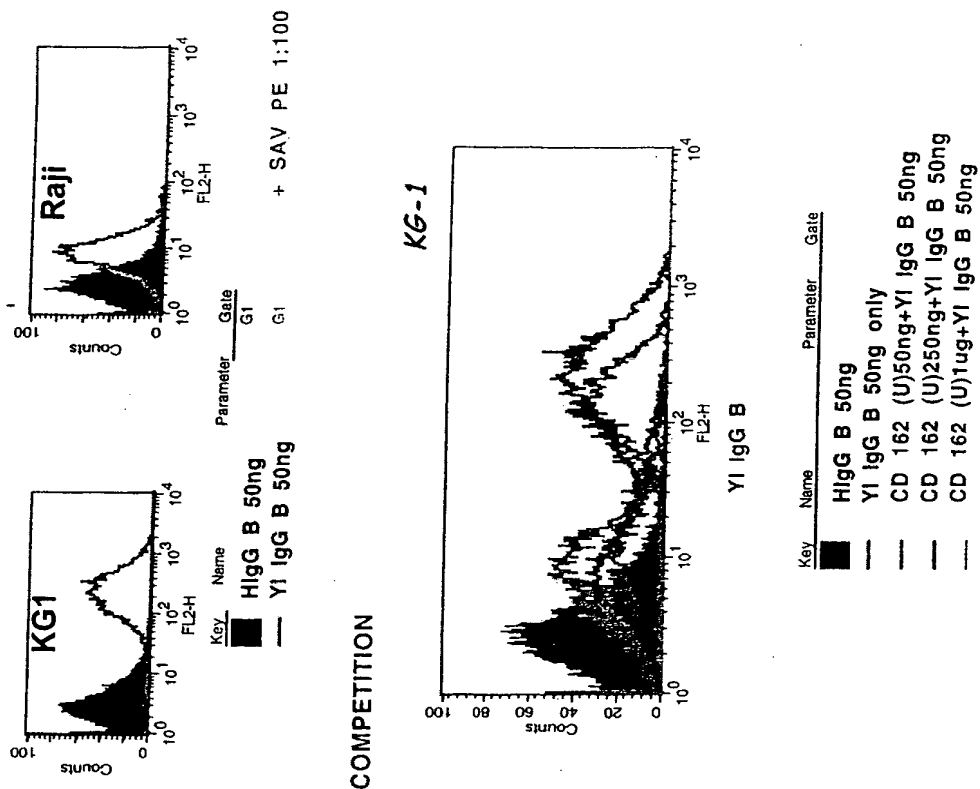


FIG. 29

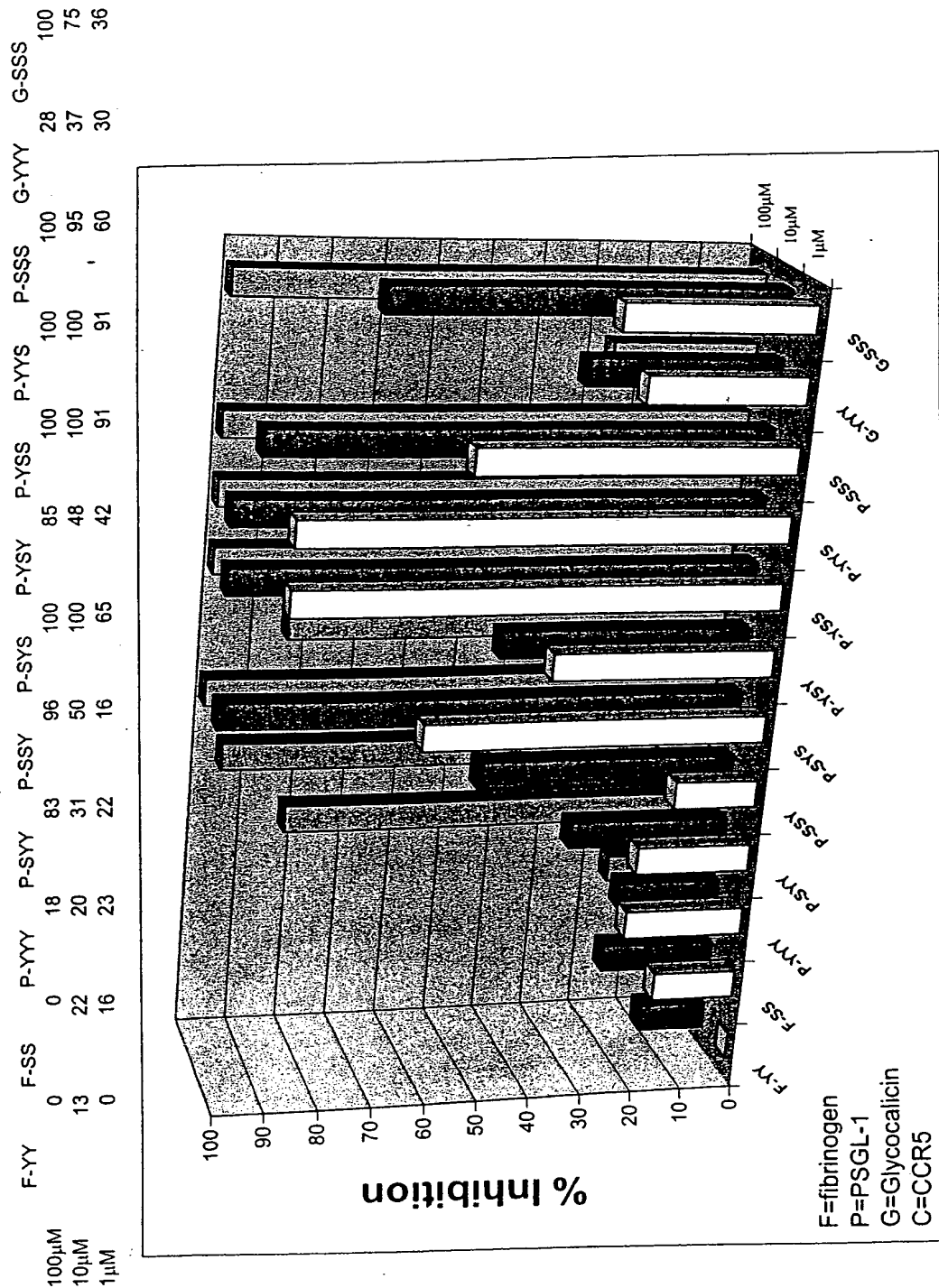




FIG. 30

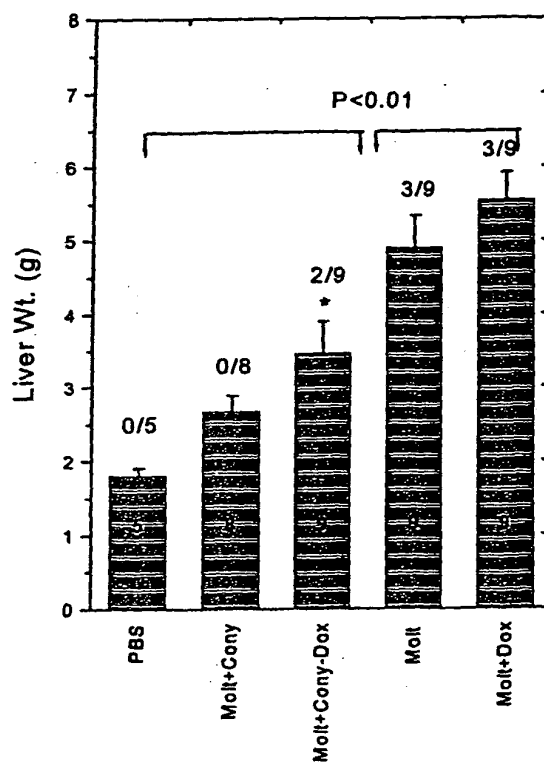




FIG. 31

*Ns were: 9 for DOX, 8 for CONY1, 7 for Y1-DOX 6 for MOLT and 5 for PBS.

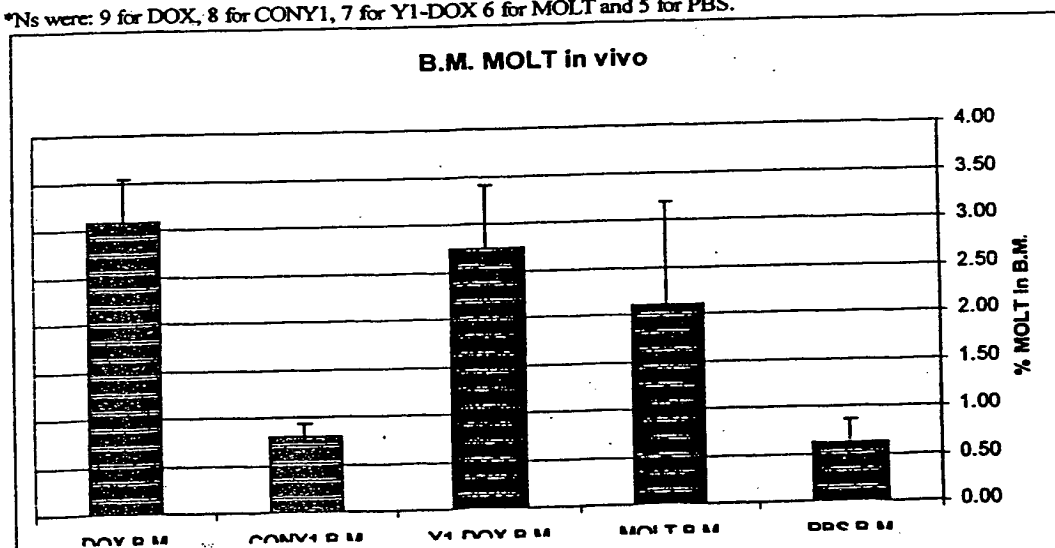
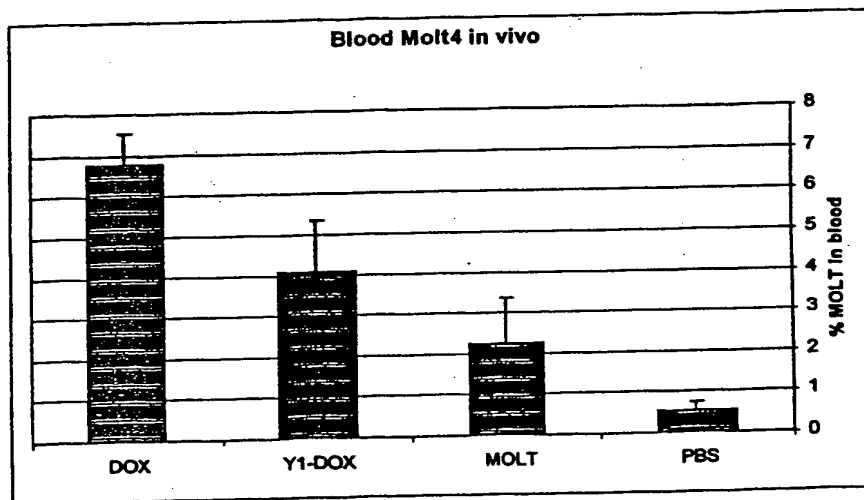




FIG. 32



**Ns were: 4 for DOX, 2 for Y1-DOX, 3 for MOLT and 3 for PBS.



FIG. 33

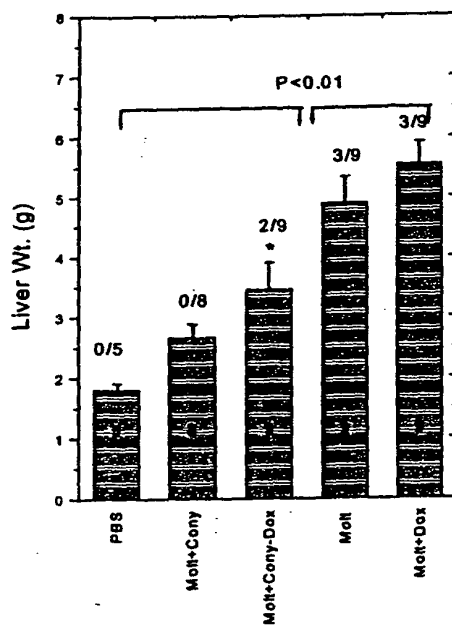
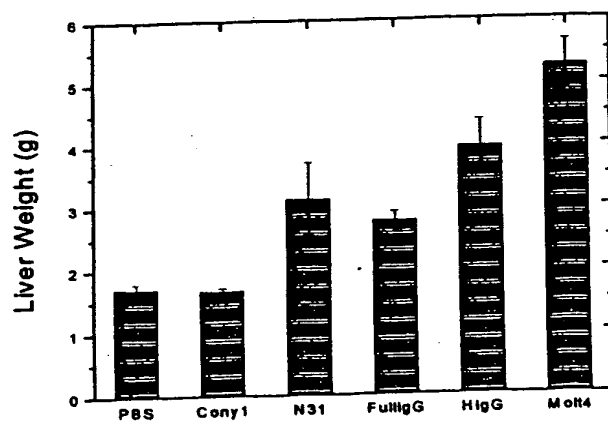




FIG. 34



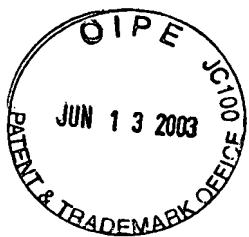


FIG. 35

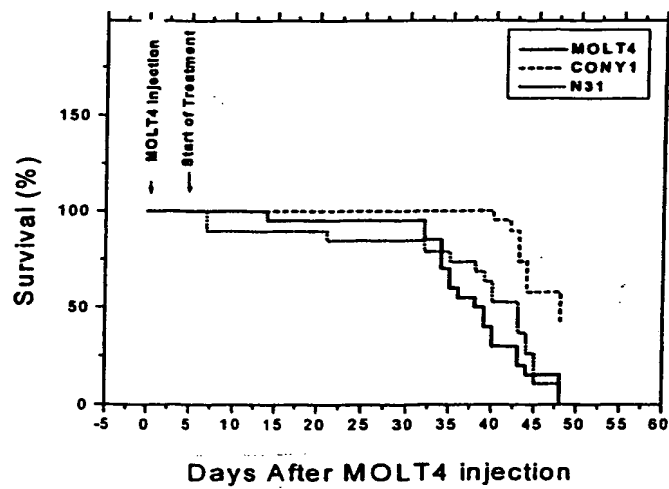




FIG. 36

***Ns were: 8 for PBS, 9 for KG1, 8 for CONY1, 11 for CONY1-DOX, 9 for DOX, 8 for 181 in vitro, 9 for Y1 in vitro and 9 for Mylotarg.

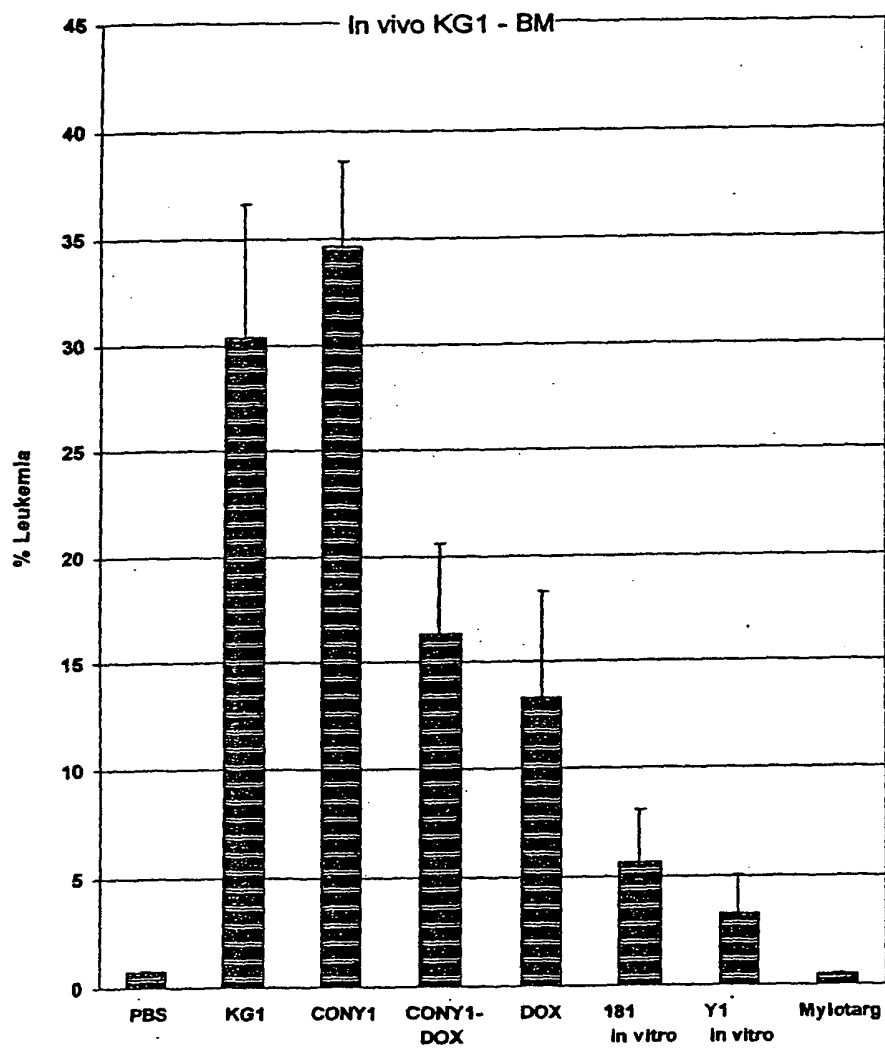
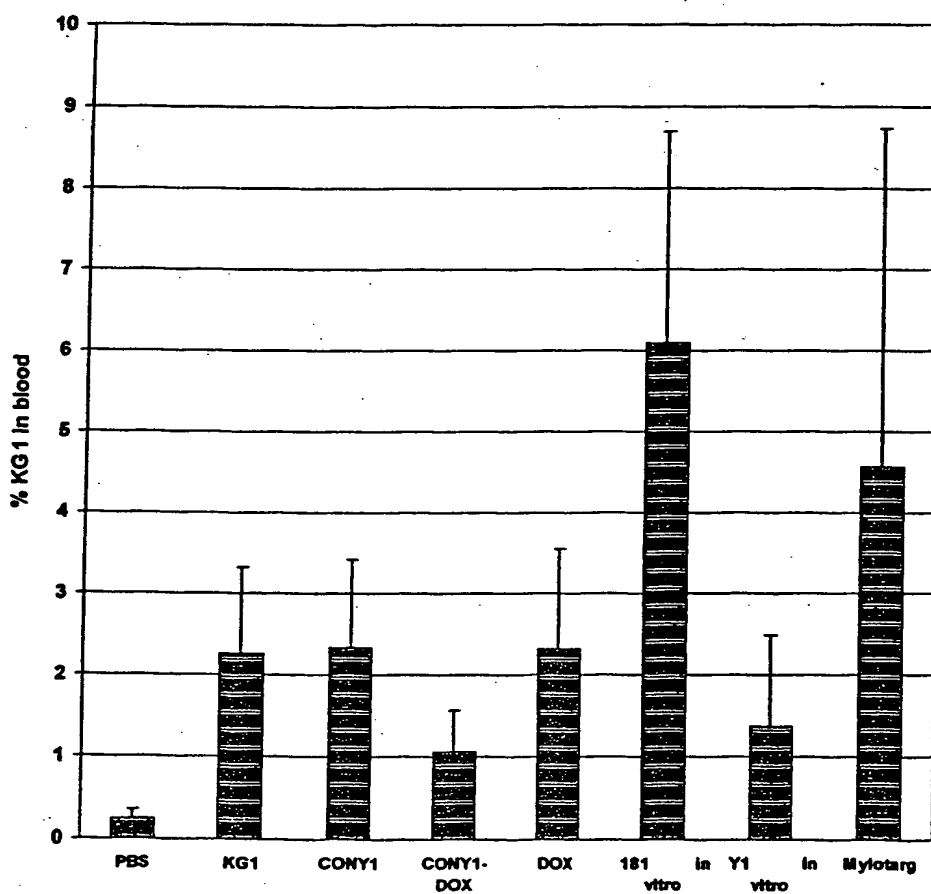




FIG. 37

In vivo KG1 - Blood



****Ns were: 8 for PBS, 9 for KG1, 8 for CONY1, 9 for CONY1-DOX, 11 for DOX (including one mice injected with 5mg/kg DOX), 7 for 181 in vitro, 8 for Y1 in vitro and 7 for Mylotarg.



FIG. 38

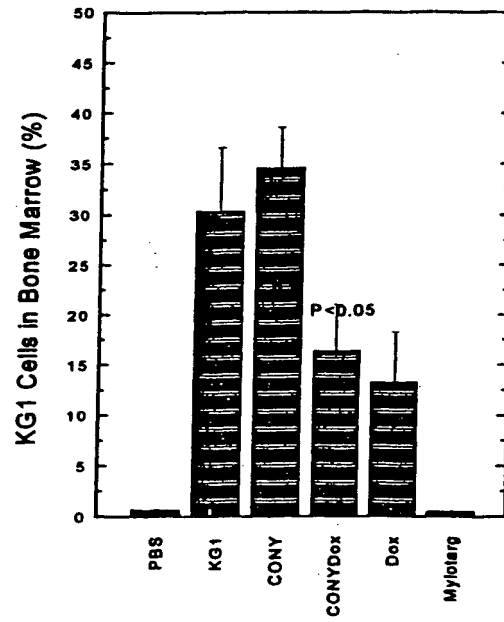
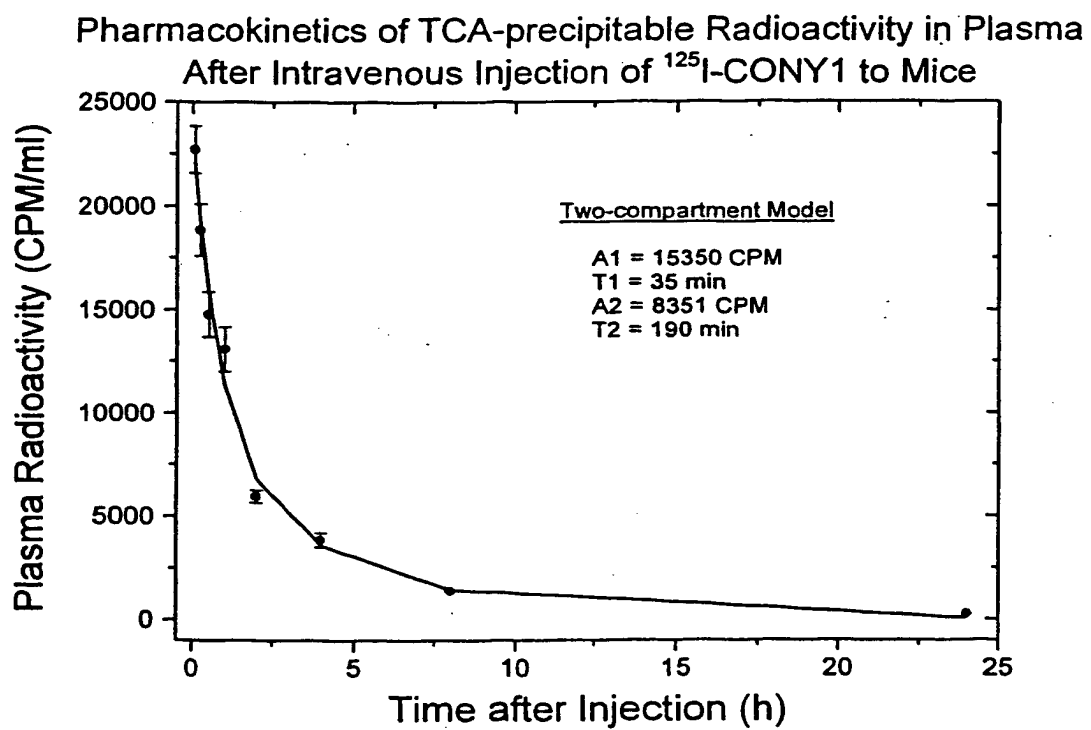




FIG. 39



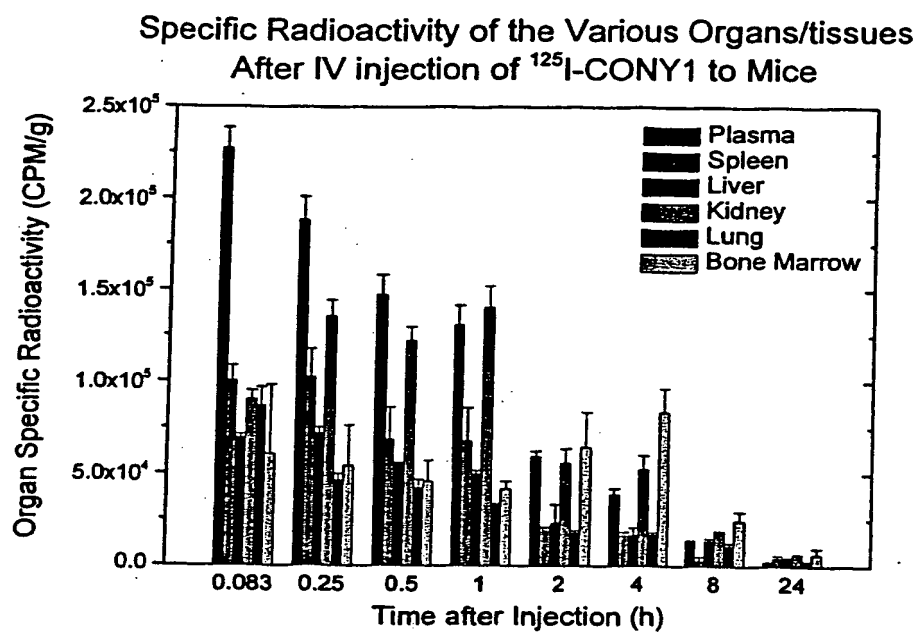




FIG. 41

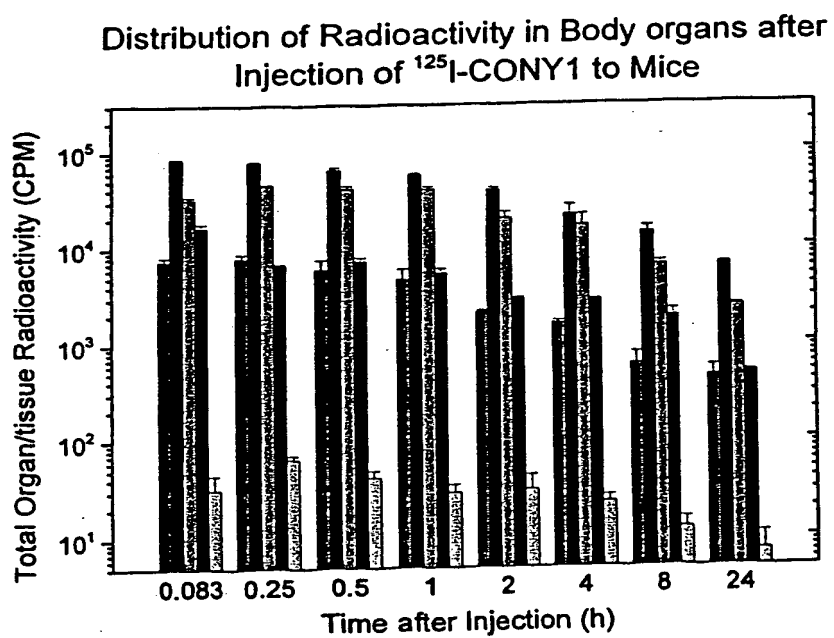




FIG. 42

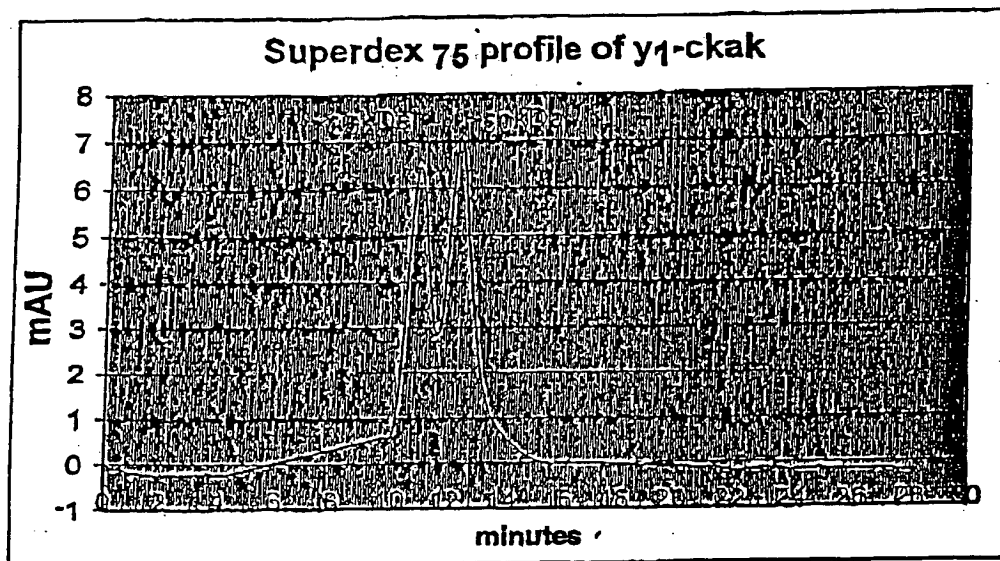


FIG. 43

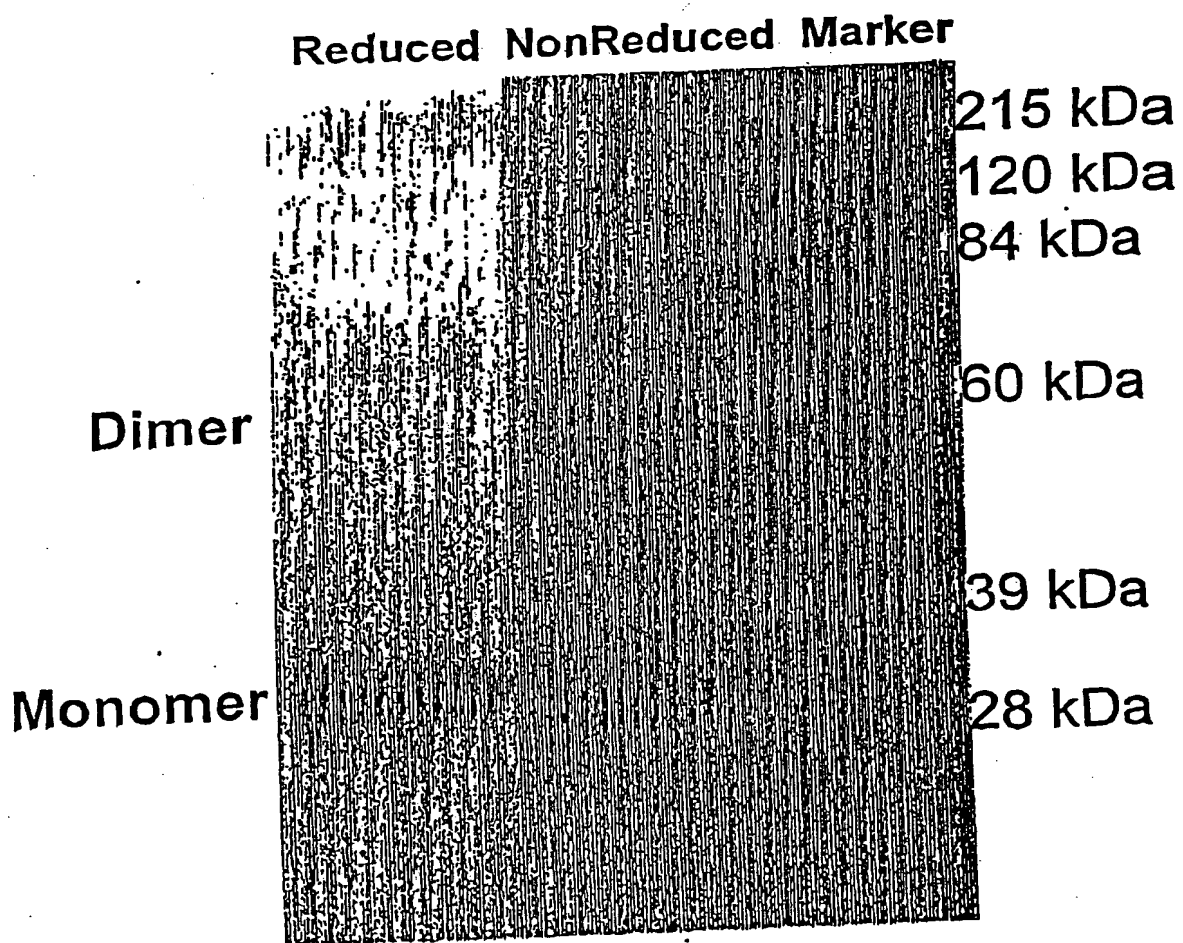




FIG. 44

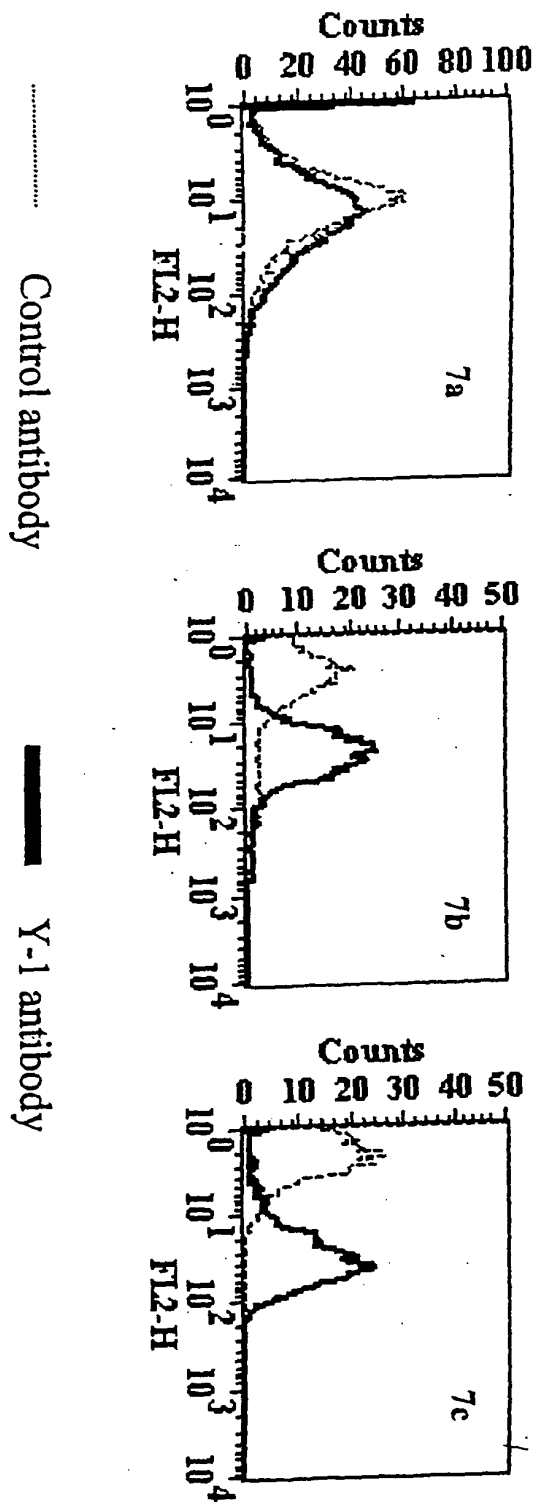
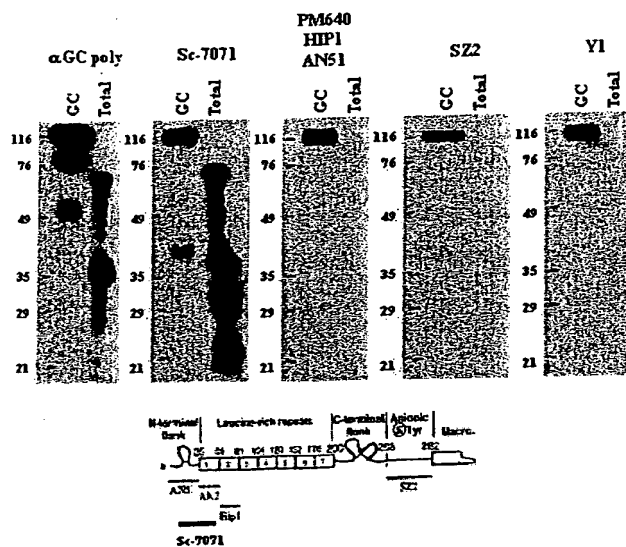




FIG. 45

Epitopes of anti-GPIb α antibodies



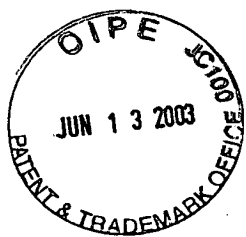


FIG. 46

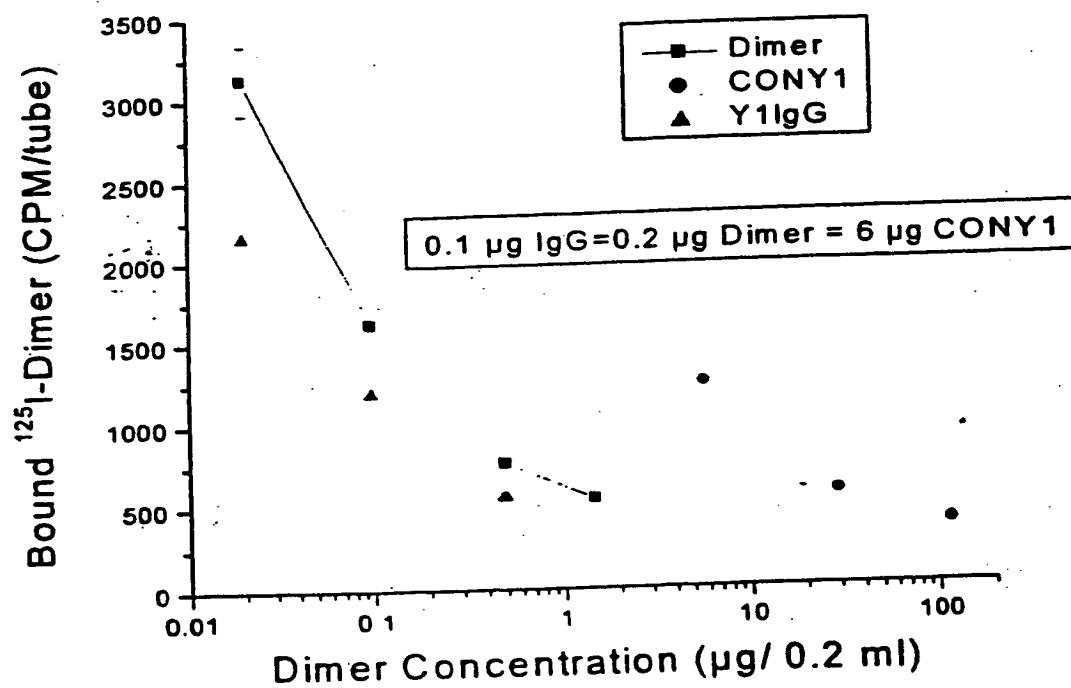




FIG. 47

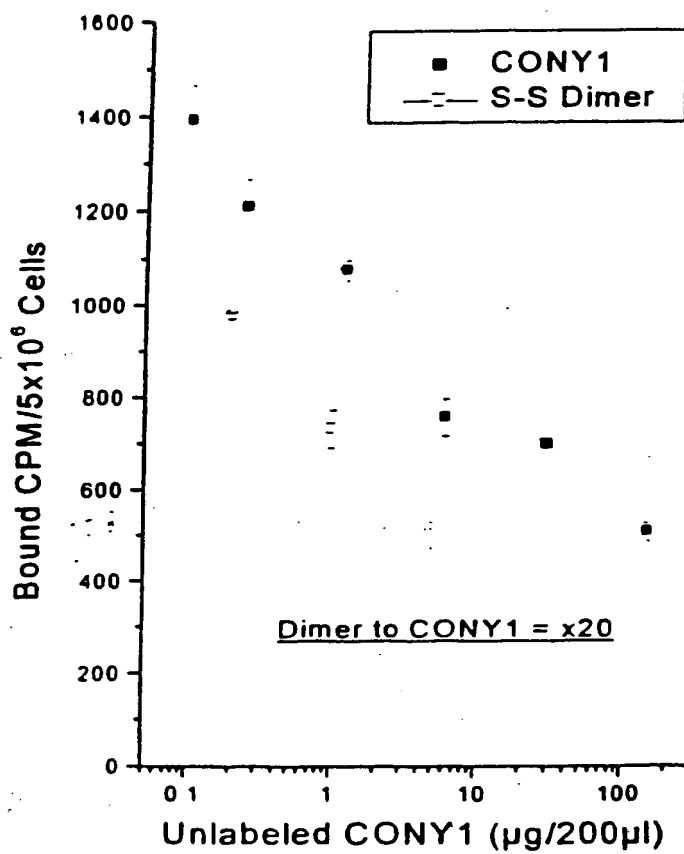




FIG. 48A: The ORF and Amino Acid Sequence of Y1-HC

SEQ ID NO: 205 (nucleic acid sequence): SEQ ID NO: 206 (amino acid sequence)

```
1      ATGGCCTGGGCTCTGCTGCTCCTOACCCTCCTCACTCAGGACACAGGGTCTGGGCCGAT
1      M A W A L L L L T L L T Q D T G S W A D
61     ATCCAGCTGGTGGAGTCTGGGGGAGGTGTGGTACGGCCTGGGGGTCCCTGAGACTCTCC
21     I Q L V E S G G G V V R P G G S L R L S
121    TGTGCAGCCTCTGGATTACCTTTGATGATTATGGCATGAGCTGGGTCCGCCAAGCTCCA
41     C A A S G F T F D D Y G M S W V R Q A P
181    GGAAGGGGCTGGAGTGGGTCTCTGGTATTAATTGGAATGGTGGTAGCACAGGTTATGCA
61     G K G L E W V S G I N W N G G S T G Y A
241    GACTCTGTGAAGGGCCGATTACCATCTCTAGAGACAACGCCAAGAAGTCCCTGTATCTG
81     D S V K G R F T I S R D N A K N S L Y L
301 -  CAAATGAACAGTCTGAGAGCCGAGGACACGGCCGTGTATTACTGTGCAAGAATGAGGGCT
101    Q M N S L R A E D T A V Y Y C A R M R A
361    CCTGTGATTGGGGCCAAGGTACCCTGGTCACCGTCTCGAGTGTCCACCAAGGGCCCA
121    P V I W G Q G T L V T V S S A S T K G P
421    TCGGTCTTCCCCCTGGCACCCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGC
141    S V F P L A P S S K S T S G G T A A L G
481    TGCCTGGTCAAGGACTACTTCCCGAACCAGGTGACGGTGTGCTGGAAGTCAAGGCGCCCTG
161    C L V K D Y F P E P V T V S W N S G A L
541    ACCAGCGCGGTGCACACCTTCCCGGCTGTCTACAGTCTCAGGACTCTACTCCCTCAGC
181    T S G V H T F P A V L Q S S G L Y S L S
601    AGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAAT
201    S V V T V P S S S L G T Q T Y I C N V N
661    CACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCCAAATCTTGTACAAAAT
221    H K P S N T K V D K R V E P K S C D K T
721    CACACATGCCCACCGTGCCAGCACCTGAACTCCTGGGGGACTGTCTAGTCTTCTCTTC
241    H T C P P C P A P E L L G G P S V F L F
781    CCCCCAAAACCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTACATGCGTGGTG
261    P P K P K D T L M I S R T P E V T C V V
841    GTGGACGTGAGCCACGAAGACCCGTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAG
281    V D V S H E D P E V K F N W Y V D G V E
901    GTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCAGTACCGTGTGGTC
301    V H N A K T K P R E E Q Y N S T Y R V V
961    AGCGTCTCACCCTCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTC
321    S V L T V L H Q D W L N G K E Y K C K V
1021   TCCAACAAAGCCCTCCCGAGCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCC
341    S N K A L P A P I E K T I S K A K G Q P
1081   OGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTC
361    R E P Q V Y T L P P S R E E M T K N Q V
1141   AGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGC
381    S L T C L V K G F Y P S D I A V E W E S
1201   AATGGGCAGCCGGAGAACAATAACAAGACCACGTCTCCCGTGGTGGACTCCGACGGCTCC
401    N G Q P E N N Y K T T S P V L D S D G S
1261   TTCTTCTCTATAGCAAGCTCACCGTGCACAAGAGCAGGTGGCAGCAGGGGAACGTCTTC
421    F F L Y S K L T V D K S R W Q Q G N V F
1321   TCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTG
441    S C S V M H E A L H N H Y T Q K S L S L
1381   TCTCTGGGTAAATGA
461    S L G K *
```




FIG. 48B: The ORF and Amino Acid Sequence of Y1-LC

SEQ ID NO: 207 (nucleic acid sequence); SEQ ID NO: 208 (amino acid sequence)

```
1      ATGGCCTGGGCTCTGCTGCTCCTCACCCTCCTCACTCAGGACACAGGGTCCTGGGCCGAT
1      M A W A L L L L T L L T Q D T G S W A D
61     GCAGAGCTGACTCAGGACCCTGCTGTGTCTGTGGCCTTGGGACAGACAGTCAGGATCACA
21     A E L T Q D P A V S V A L G Q T V R I T
1212   TGCCAAGGAGACAGCCTCAGAAGCTATTATGCAAGCTGGTACCAGCAGAAGCCAGGACAG
41     C Q G D S L R S Y Y A S W Y Q Q K P G Q
181    GCCCCTGTA CTGTGTCATCTATGGTAAAAACAACCGGCCCTCAGGGATCCCAGACCGATT
161    A P V L V I Y G K N N R P S G I P D R F
241    TCTGGCTCCAGCTCAGGAAACACAGCTTCCTTGACCATCACTGGGGCTCAGGCGGAAGAT
81     S G S S S G N T A S L T I T G A Q A E D
301    GAGGCTGACTATTACTGTAAC TCCCGGGACAGCAGTGGAACCATGTGGTATTCGGCGGA
101    E A D Y Y C N S R D S S G N H V V F G G
361    GGGACCAAGCTGACCGTCTAGGT CAGCCCAAGGCTGCCCCCTCGGTCACTCTGTTC
121    G T K L T V L G Q P K A A P S V T L F P
421    CCCTCCTCTGAGGAGCTTCAAGCCAACAAGGCCACACTGGTGTGTCTCATAAGTGACTTC
141    P S S E E L Q A N K A T L V C L I S D F
481    TACCCGGGAGCCGTGACAGTGGCCTGGAAGGCAGATAGCAGCCCCGTCAAGGCGGAGTG
161    Y P G A V T V A W K A D S S P V K A G V
541    GAGACCACACACCTCCAAACAAGCAACAACAAGTACGCGGCCAGCAGCTACCTGAGC
181    E T T T P S K Q S N N K Y A A S S Y L S
601    CTGACGCCTGAGCAGTGGAAGTCCCACAAAAGCTACAGCTGCCAGGTACGCATGAAGGG
201    L T P E Q W K S H K S Y S C Q V T H E G
661    AGCACCGTGGAGAAGACAGTGGCCCCTACAGAATGTTTCATGA
221    S T V E K T V A P T E C S *
```



FIG. 49

1	11	21	31	41	51	
1	EVQLVESGGG	LVQPGGSLRL	SCAASGFTFS	SYAMSWVRQA	PGKGLEWVSA	ISGSGGSTYY 60
61	ADSVKGRFTI	SRDNSIKNTLY	LOMNSLRAED	TAVYYCARVA	KTLMRQYSLW	GQGTLVTVSR 120
121	GGGSGGGGGS	GGGGSSELTQ	DPAVSVALGQ	TVRITCGGDS	LRSYIASWYQ	QKPGQAPVLV 180
181	IYGKNNRPSG	IPDRFSGSSS	GNTASLTITG	AQAEDADYY	CNSRDSSGNH	VVFGGGTKLT 240
241	VLGAAAEQKL	ISEEDLNGAA				



FIG. 50

	10	20	30	40	50	60
1	AttTattACTC	gGGGCCAGC	CgGCCAGC	CGAGGTGCAG	CTGGTGGAGT	CTGGGGGAGG
3	L L L A A Q P A M A	E V Q L V E S G G G				
	70	80	90	100	110	120
1	CTTGGTACAG	CCTGGGGGGT	CCCTGAGACT	CTCCTGTGCA	GCCTCTGGAT	TCACCTTTAG
3	L V Q P G G S L R L S C A A S G F T F S					
	130	140	150	160	170	180
1	CAGCTATGCC	ATGAGCTGGG	TCCGCCAGGC	TCCAGGGAAG	GGGCTGGAGT	GGGTCTCAGC
3	S Y A M S W V R Q A P G K G L E W V S A					
	190	200	210	220	230	240
1	TATTAGTGGT	AGTGGTGGTA	GCACATACTA	CGCAGACTCC	GTGAAGGGCC	GGTTCACCAT
3	F S G S G G S T Y Y A D S V K G R F T I					
	250	260	270	280	290	300
1	CTCCAGAGAC	AATTCOAAGA	ACACGCTGTA	TCTGCAATG	AACAGCCTGA	GAGCCGAGGA
3	S R D N S K N T L Y L Q M N S L R A E D					
	310	320	330	340	350	360
1	CACGGCCGTG	TATTACTGTG	CAAGACCGG	CGCAGTCTTT	AGGGTAACT	GGGGCCAGG
3	T A V Y Y C A R T G Q S I K R S W G Q G					
	370	380	390	400	410	420
1	TACCCTGGTC	ACCGTGTGGA	GAGGTGGAGG	CGGTTCAggC	GGagGTGgCT	CTGGCGGTGG
3	T L V T V S R G G G G S G G G G S G G G					
	430	440	450	460	470	480
1	CGGATGCTCT	GAGCTGACTC	AGGACCCTGC	TGTGTCTGTG	GcCTTGGGAC	AgACAGTCAG
3	G S S E L T Q D P A V S V A L G Q T V R					
	490	500	510	520	530	540
1	GATCACATGC	CAAGGAGACA	GCCTCAGAAG	CTATTATGCA	AGCTGGTACC	AGCAGAAGCC
3	I T C Q G D S L R S Y Y A S W Y Q Q K P					
	550	560	570	580	590	600
1	AGGACAGGCC	CCTGTACTTG	TCATCTATGG	TAAAAACAAC	CGGCCCTCAG	GGATCCCAGA
3	G Q A P V L V I Y G K N N R P S G I P D					
	610	620	630	640	650	660
1	CGGATTCCTT	GGCTCCAGCT	CAGGAAACAC	AGCTTCCTTG	ACCATCACTG	GGGCTCAGGC
3	R F S G S S S G N T A S L T I T G A Q A					
	670	680	690	700	710	720
1	GGAAGATGAG	GCTGACTATT	ACTGTAACTC	CCGGGACAGC	AGTGGTAACC	ATGTGGTATT
3	E D E A D Y Y C N S R D S S G N H V V F					
	730	740	750	760	770	780
1	CGGCGGAGGG	ACCAAGCTGA	CCGTCCTAGG	TGCGGCCGCA	GAACAAAAC	TCATCTCAGA
3	G G G T K L T V L G A A A E Q K L I S E					
	790	800	810	820	830	840
1	AGAGGatCTG	AatGGGGCCG	CACTGACTG	TtGAATTTT	TAAGTAAcC	T
3	E D L N G A A * N C * I F * V N					

1/16 SEQ ID NO: 210



FIG. 51

Sequence of Y1-Biotag (SEQ ID NO: 211)

1 MEVQLVESGG GVVRPGGSLR LSCAASGFTF DDYGMSWVRQ
41 APGKGLEWVS GINWNGGSTG YADSVKGRFT ISRDNAKNSL
81 YLQMNSLRAE DTAVYYCARM RAPVIWGQGT LVTVSRGGGG
121 SGGGGSGGGG SSELTDPAV SVALGQTVRI TCQGDSLRSY
161 YASWYQQKPG QAPVLVIYGK NNRPSGIPDR FSGSSSGNTA
201 SLTITGAQAE DEADYYCNSR DSSGNNVVFG GGTKLTVLGG
241 GGLNDIFEAQ KIEWHE



FIG. 52

Y1-cys-kak scFv (SEQ ID NO. 212)

I MEVQLVESGG GVVVRPGGSLR LSCAASGFTF DDYGMSWVRQ
APGKGLEWVS GINWNGGSTG 60

61 YADSVKGRFT ISRDNAKNSL YLQMNSLRAE DTAVYYCARM
RAPVIWGQGT LVTVSRGGGG 120

121 SGGGSGGGG SSELTDPAV SVALGQTVRI TCQGDSLRSY
YASWYQQKPG QAPVLVIYGK 180

181 NNRPSGIPDR FSGSSSGNTA SLTITGAQAE DEADYYCNSR
DSSGNHVVFG GGTKLTVLGG 240

241 GGCKAK